"Some Photo-reactions of Chlorophyll and Related Molecules".

Thesis presented for the degree of Doctor of Philosophy by

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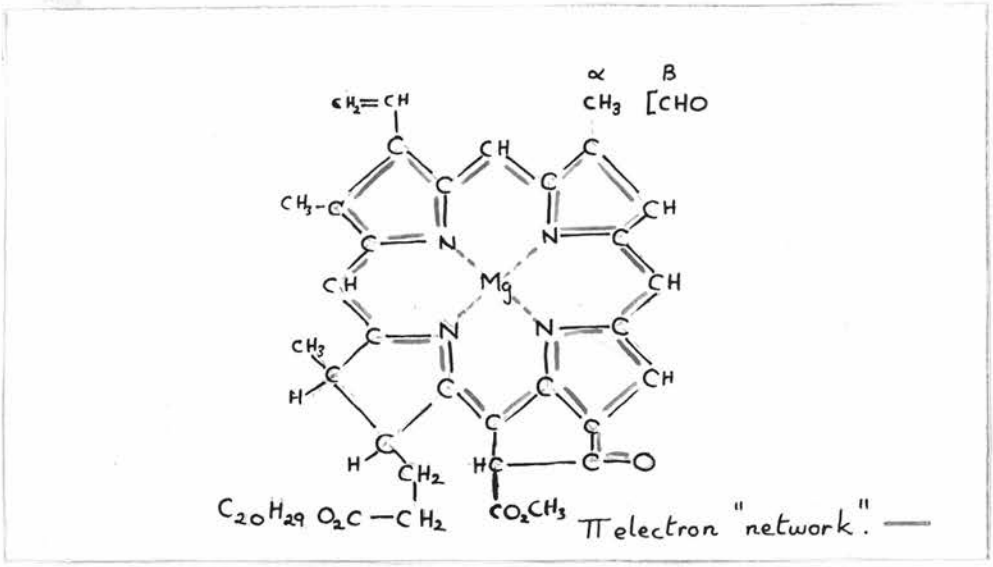
Introduction

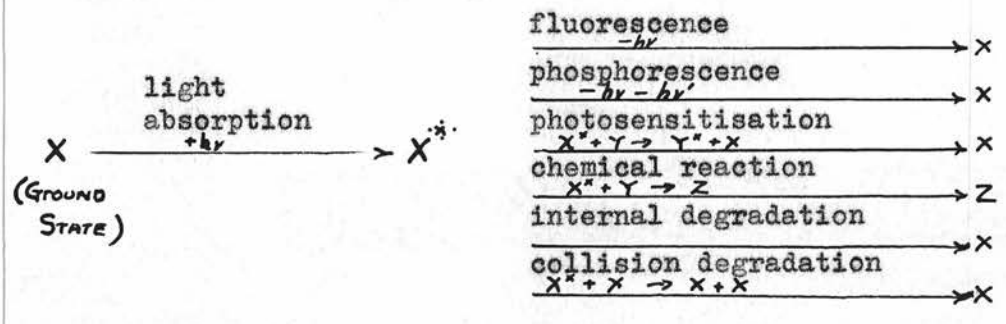
The colouring pigment of the plant leaf contains a mixture of several polyenic compounds, the carotenes, and two chlorin derivatives, chlorophylls α and β. It is in this highly complex mixture that the first stages of the photosynthetic process take place, viz., the absorption of solar radiation by the green leaf. The most active light-absorbing entities are the chlorophylls themselves.



This formula gives a truer picture of the molecule as an extensive conjugated lattice, than any of the separate Kekulé formulae.

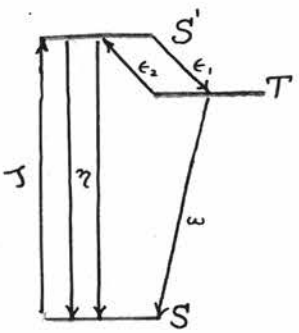
In vitro, the molecule is highly susceptible to degradation, the central magnesium atom, for example, is readily replaced by hydrogen (phaeophitinisation)(1), or by other metals. Photo-bleaching, both reversible (2) and irreversible (3) can be carried out and oxygen appears to be a participant in such reactions (4). The chlorophylls are also highly reactive as photosensitisers in the photo-oxidation of other molecules. A primary problem in the study of photosynthesis is to decide whether chlorophyll is merely a photosensitiser or is actually involved in the reaction sequence chemically and later regenerated.

The absorption of light by a polyenic molecule is directly related to the permissible energy levels of the electrons in the molecule. In certain cases it has been found possible to calculate such energy levels theoretically (5) using a simple electron gas model. On absorption of light the molecule is raised to a higher energy level. Such a level is not stable and the excess energy may be lost in several ways with reversion to the lower energy state.



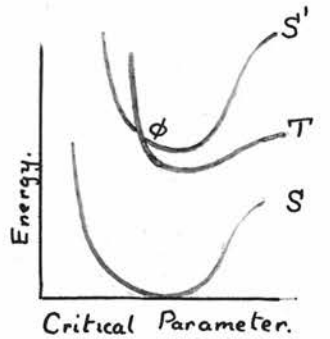
The phenomenon of phosphorescence or prolonged luminescence after illumination, seemed at first sight to bear very little relation to the other photo-processes listed. It occurs, generally, when certain dyestuffs are illuminated in the solid state (6). The "afterglow" consists of two bands, an α band, identical in wavelength with the normal fluorescence of the dyestuff, and a β band, of somewhat longer wavelength (7). The α band predominates at high temperatures, the β band at low temperatures. The phosphorescence of fluorescein in boric acid is typical, changing from yellow, through green, to blue, as the temperature is raised (8).

The phenomenon was first given a theoretical interpretation by Jablonski (9). This interpretation, though modified slightly, has not been basically altered (10). The theory postulates a metastable energy level T for the light-absorbing molecule, "metastable" implying a stability to loss of energy by a radiative process. The molecule has ground state S and fluorescent state S'



Fluorescence is due to absorption of light ζ, S→S', and re-emission of energy η, S'→S. A molecule in state S' may however enter state T by some process ϵ_1, and from this state a slow "leak" of energy occurs, either by radiation ω, T→S, or by thermal excitation to S' ϵ_2, followed by normal fluorescence η. T→S accounts for β phosphorescence, T→S'→S for α phosphorescence. α predominates over β at high temperatures, hence the variation of type of phosphorescence with temperature.

Further investigations of the phenomenon led to the demonstration that the phosphorescent state T corresponds to a triplet, paramagnetic state of the molecule (11). The best interpretation of the phenomenon is in terms of potential energy curves (12). A molecule absorbing light is raised to an upper state S'. In the solid phase, particularly at low temperatures, a rapid "cascade" down to the lower vibrational levels of S' occurs and at ϕ there is a certain amount of "mixing" with the triplet state T, i.e. S' ⇌ T. A further loss of vibrational energy may well leave the molecule in the long-lived energy level T.

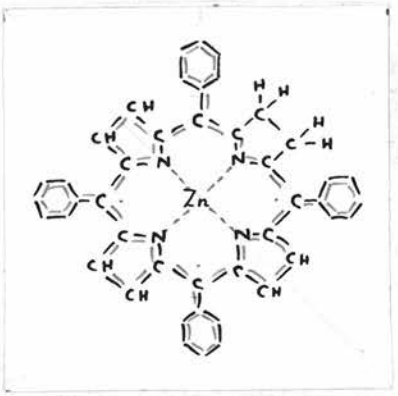


The most important facts from our point of view are :-

(a) The triplet state is frequently attained on illumination of dyestuffs in the solid phase.

(b) A molecule in this state is a di-radical and a susceptibility to attack by oxygen, itself a di-radical, would by no means be unexpected.

Attempts to demonstrate the phosphorescence of the chlorophylls have met with varying success (13,14). In the case of the simpler chlorins, a marked phosphorescence has been found in several cases. A study of the photo-oxidation of these chlorins, e.g. zinc-tetra-phenyl chlorin, gave some unusual results in the kinetics of the reaction. Without



going into details, it was found that the lifetime of the phosphorescent molecule at the temperature of the reaction admirably suited the kinetics. The thermal "destruction" of state T at high temperatures explains the negative temperature coefficient of reaction (15,16).

The investigation of reactions in solution, particularly where radicals of any description may be formed, has one great disadvantage in that solvent molecules may be attacked. Reactions carried out between gaseous and solid phase reactants are, therefore, to be preferred, but in the case of photo-reactions are normally extremely slow and very sensitive pressure measurements must be made. The ideas behind the present technique of investigating chlorophyll degradation were multifold :-

(a) It has been suggested that, in the chloroplasts, chlorophyll is present in the absorbed phase (17).

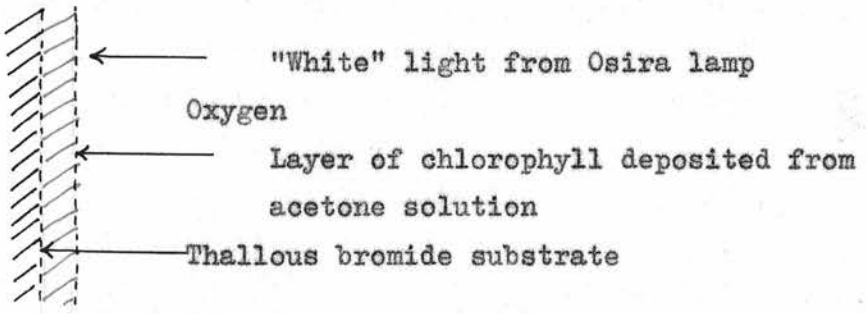
(b) Chlorophyll desensitises thallous bromide plates (18).

(c) If the reaction involved a triplet state molecule it might show up in the kinetics.

(d) It was hoped that a simplification of the reaction mechanism would be obtained in the solid phase.

Photo-sensitisation can occur in the solid state as well as in the liquid and gas phases. In a photo-conducting solid, electrons which, by absorption of light, have gained sufficient energy to reach the conduction band of the solid, are non-localised, i.e. are free to move through the crystal lattice and are not associated with any particular atom or molecule in the lattice. When a dyestuff is deposited on the surface of such a solid it is possible for a transfer of such electrons to occur between the dyestuff and the photo-conductor in such a way that the dyestuff molecule is raised to a reactive electronic state. It is thus possible for the semiconductor to act as a catalyst for the photochemical reaction of the dyestuff.

The reaction between chlorophyll, in the solid phase, and oxygen has been followed in the present investigations, by pressure measurements using a thermostated reaction vessel-Bourdon gauge system. Thallous bromide acts as a sensitiser for the reaction and the system is indicated schematically below. It is possible to determine, by a rate extrapolation method, the ratio moles of gas phase reacting (as a pressure change) to moles of chlorophyll. This ratio will be called $\varnothing$ throughout the thesis.



The work carried out by previous workers in this field had led to the following results (19,20,21).

(a) during the normal photo-oxidation at high pressures (100mm.) $\phi = 1$

(b) evacuation of the system causes apparent reversal of the normal reaction with attainment of the original, or at least an increased, rate of reaction on admission of oxygen and re-illumination.

(c) the oxidised film gave a colour reaction with ferrous thiocyanate in methanol, quantitatively equivalent to one mole of hydro-peroxide formation per mole of normal pressure decrease. The exact nature of the entity giving the colour reaction has not been confirmed as peroxidic.

(d) an apparent dissociation pressure of oxygen was found by extrapolation of the rate/pressure curve to zero rate.

The mechanism adopted in view of these facts was the relatively simple one :-

$$Chl + h\nu \rightarrow Chl^{*} \qquad (1)$$

$$Chl^{*} + O_2 \rightarrow ChlO_2r \qquad (2)$$

$$ChlO_2r \rightleftharpoons Chl + O_2 \qquad (3)$$

$$ChlO_2r \xrightarrow{(h\nu ?)} ChlO_2i \qquad (4)$$

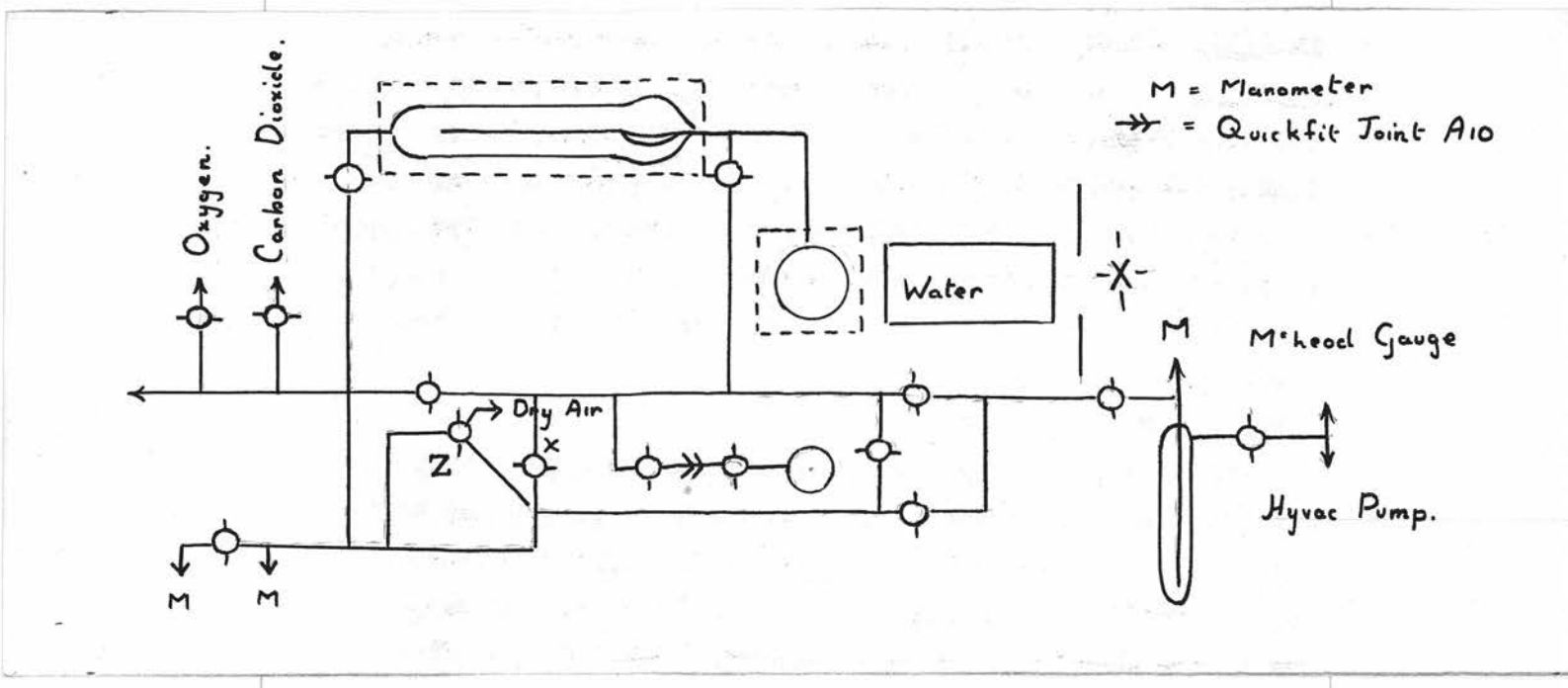
Chl, chlorophyll in the ground state.
Chl, chlorophyll in some excited state.
$ChlO_2r$, a dissociable oxidised product.
$ChlO_2i$, an undissociable oxidised product.

These four stages accounted for many of the experimental results, the main anomalies being the following :-

(a) When the reaction is carried out in the presence of adsorbents such as phosphoric oxide ϕ increases well above unity and the participation of at least one other gas besides oxygen is indicated.

(b) A small uptake of oxygen was observed below the apparent dissociation pressure of the oxidised products.

It was thought that a consecutive reaction scheme was the best approach to the problem and the present research was designed to verify the details of the original research, to isolate the intermediate products and to clarify the reaction mechanism.


M = Manometer
= Quickfit Joint A10
Oxygen.
Carbon Dioxide.
Water
M
McLeod Gauge
Dry Air
Z
X
Hyvac Pump.
M
M

Experimental.

: Apparatus.

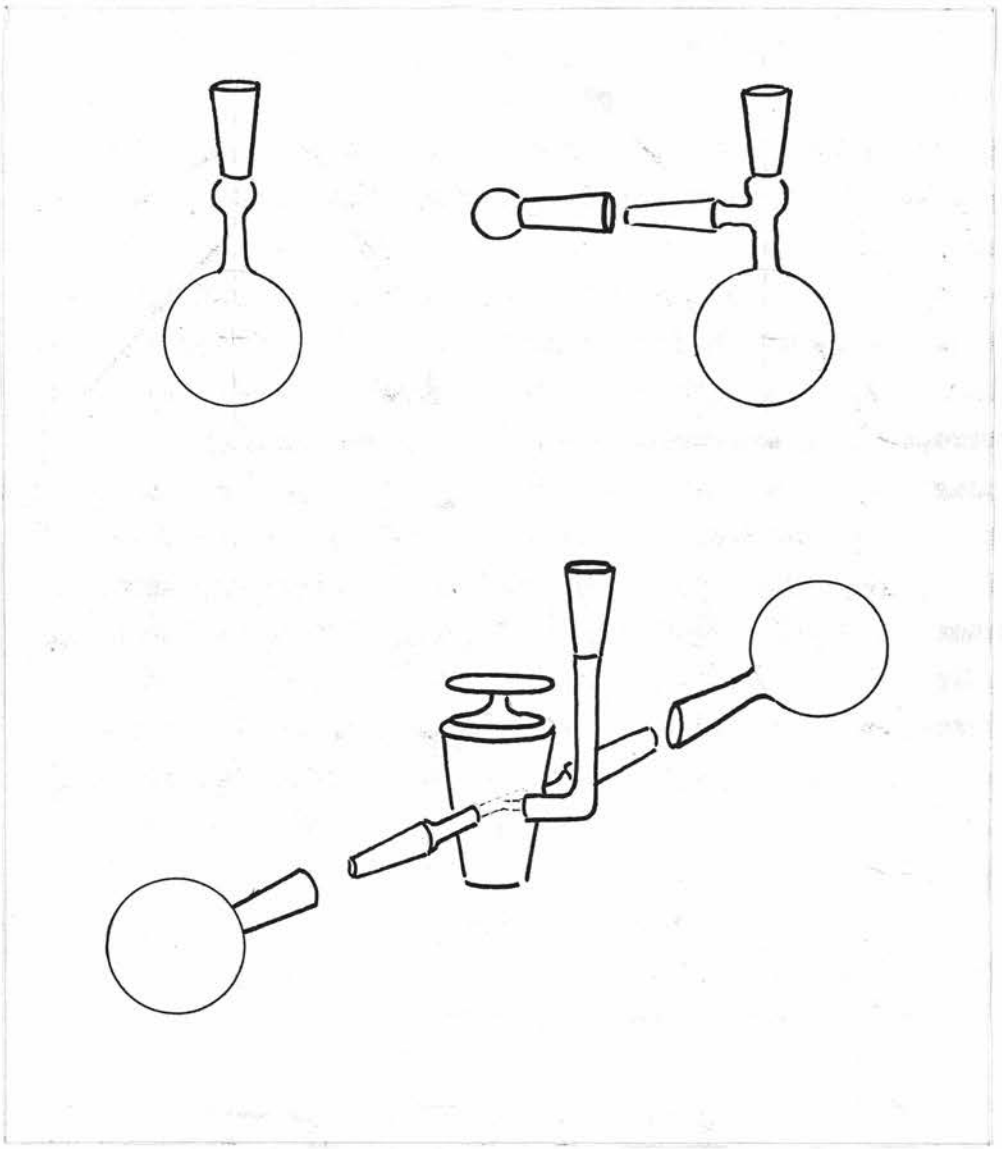
The apparatus used throughout this work consisted of a thermostated reaction vessel-Bourdon gauge system, as indicated opposite. It was constructed entirely of soda-glass with the exception of the reaction vessels which were of "Pyrex". The reaction vessels were connected to the gauge by an A(10) cone and socket joint. All taps and socket joints were lubricated with Apiezon grease "L", which was found to be not only vacuum-tight, but also in certain experiments water-tight, even at 25° C. The whole apparatus could be evacuated to a pressure of 10^{-3} mm. of mercury, by means of a "Speedivac" pump. The trap shown was always surrounded by liquid oxygen or nitrogen during evacuation to minimise or prevent back diffusion of pump oil vapour.

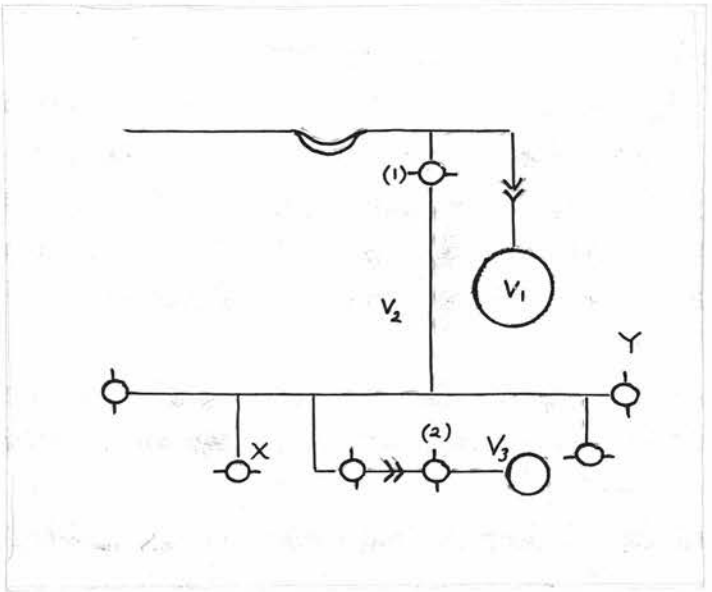
: Thermostating System.

Pressure changes during the reaction were generally slight so that extremely sensitive Bourdon gauges had to be used. These, in turn, necessitated very rigid temperature control over the gas present, both in the reaction vessel and in the gauge and jacket. This was achieved by a flow system. Water from a thermostat tank was pumped to a still-head from which it flowed in a divided stream, over the reaction vessel and the gauge, through jackets, indicated by a dotted line, and then back to the tank. All parts of this flow system were lagged with felt and asbestos string. The thermostat tank was maintained at 25 ± ·01° C. by a large, thin glass, chloroform-mercury control in conjunction with a "Sunvic" relay and heater.

: Pressure measurement and gauge calibration.

Pressure variation during the reaction was followed by direct observation of the movement of the gauge pointer, through a telescope fitted with a graduated eyepiece. The calibration of the gauge was carried out as follows. Dry air was admitted to the apparatus to a pressure p as indicated on




(1)
V_1
V_2
Y
X
(2)
V_3

the manometer. The opposite sides of the gauge were then separated by closing tap X. The right hand side of the gauge was then evacuated, via tap Y, until the gauge pointer reached a suitable position on the scale. The left hand side of the gauge was then evacuated via tap Z, causing the gauge pointer to sweep across a number of scale divisions n with corresponding manometer change Δp. Δp was usually insignificant for one such operation so that a series of such sweeps were carried out. Σn was equated to Δp and the sensitivity thus found. The sensitivity of such gauges was generally 0.002 - 0.004mm. / 0.1 scale division. During the entire operation water, at 25°C., was run through the thermostating system. Determinations of sensitivity were always repeated several times. No significant variation of sensisivity with absolute pressure, was ever detected.

Ex.	Δp	Σn	Sensitivity
	0.66mm.	300.3	0.00220
	0.64cm.	300.1	0.00212
	0.78cm.	360.9	0.00216

Mean 0.00216mm. / 0.1 scale division.

: Reaction vessel volume calibration.

Reaction vessels were spherical and were attached to the apparatus by a quickfit A(10) joint. In the case of experiments with adsorbents, a special, small volume, side tube contained the adsorbent material. The three-way tap reaction vessel shown is of general utility in that it provides

(a) a method of sampling the gas phase during a reaction.
(b) a method of connecting in various reagents during a reaction
(c) a method of testing for separate gas and gas-solid reactions.

The volume of the reaction space was found as follows. The apparatus was filled with dry air to a pressure p1, taps 1 and X closed, and the volume V2 + V3 evacuated via tap Y. Tap 2 was then closed and the air in reaction space V1 expanded into V2, pressure being reduced on the opposite side of the gauge by evacuation via tap Z, until a pressure p2 was reached at equilibrium. Expansion was similarly carried out into the calibration volume V3 until equilibrium was reached at a pressure p3. The gauge was used as a null point indicator throughout.

$$p1V1 = p2(V1 + V2) = p3(V1 + V2 + V3)$$

$$p1p3V1 = p2p3V1 + p2p3V2$$

$$p1p2V1 = p2p3V1 + p2p3V2 + p2p3V3$$

$$\therefore V1 = p2p3V3 \,/\, p1(p2 - p3)$$

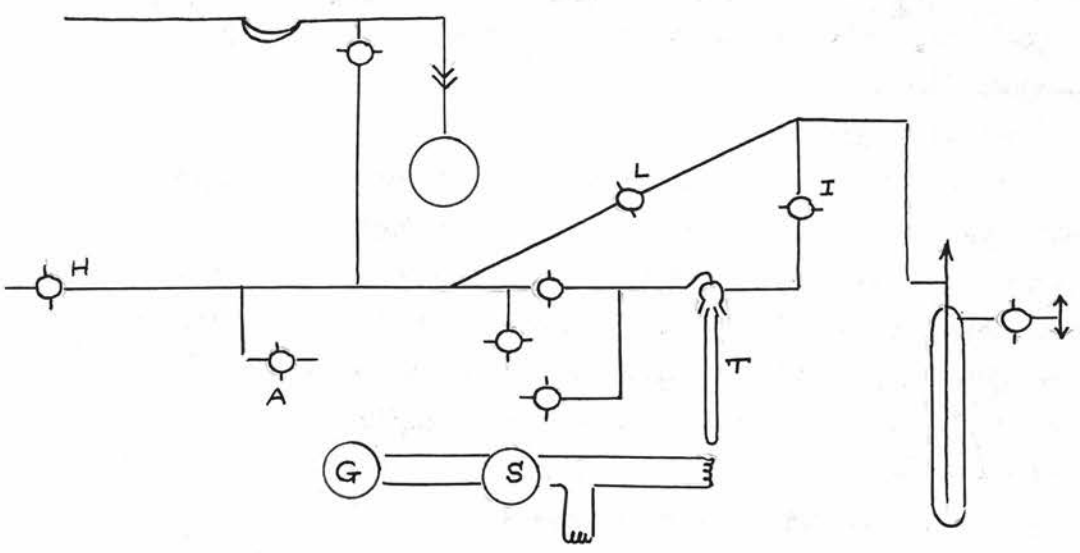
V3 was found by weighing the detachable bulb empty, then filled with water, up to and including the capillary portion of the tap key (tap 2). Determinations of V1 were carried out several times, a mean value of the results, which generally lay in a range V - 0.2mls. to V + 0.2mls., being taken.

Ex.	p1	p2	p3	V
	52.78	35.36	17.61	58.03ccs.
	50.55	33.91	16.86	57.91ccs.

Pressures p1, p2, and p3 are in cms. of mercury.

: Ozonisation experiments.

A special side-tube of the type shown, in conjunction with the three-way tap arrangement, was used to study the effect of ozone on the gases produced during the reaction. This attachment was made of soda-glass, the electrodes being of thin platinum wire. The glass-platinum joint was liberally covered with "picien" wax to avoid leaks. A spark discharge was produced by an induction coil.


L
I
H
A
T
G
S

: Micro gas analysis.

It was found possible to adapt the apparatus for micro-gas analysis, using a micro-distillation method. A micro-trap T was fitted to the evacuation line as shown. At the end of a normal "run" this trap was surrounded by liquid oxygen and the gas phase pumped slowly through the trap. Over-rapid pumping resulted in condensables being carried through as a fine spray. The contents of the trap, after removal of liquid oxygen, were allowed to evaporate slowly, the trap being surrounded by an insulating jacket, into the reaction vessel and gauge. Typical pressure/time curves were obtained for CO_2/ H_2O mixtures. A thermocouple-galvanometer system enabled pressure/f(temperature) curves to be obtained, where f(temperature) is a galvanometer reading dependant on the trap temperature. These served to identify gaseous reaction products by the typical values of f(T) at which pressure changes occurred. The bypass (L) serves to evacuate any special reactants connected at A without passing through tap T, so that by suitable choice of adsorbent materials the apparatus can provide a valuable method of routine analysis on semi-micro and micro gas samples (22).

: Light source.

Throughout all the experimental work an Osira mercury lamp was used. (125W./230V.) Light from this source was passed through a cylindrical water tube with plane glass (soft) ends. This provided partial collimation of the beam while at the same time removing ultra-violet and infra-red radiation from the beam. No light of wavelength < 3650A was thus transmitted.

: Purification and storage of gases.

Oxygen, from a commercial cylinder, was passed slowly through phosphoric oxide and a liquid oxygen trap and stored in a previously evacuated glass bulb. Carbon dioxide was admitted directly through phosphoric oxide to a bulb and purified by freezing out in liquid oxygen and pumping off the

residual impurities. This process was repeated several times. Water vapour was admitted directly to the reaction vessel by means of a tube containing distilled water attached at the quickfit joint normally used to attach the calibrated bulb V3. Tap X was kept closed during the admission of water vapour.

: Preparation of chlorophyll solutions.

As a source of chlorophyll, two commercial "chlorophyll" pastes supplied by J.F.McFarlan and Co., were used. Both of these were alcohol/acetone extractions. The first of the samples, which had been used by previous workers in this department, in similar studies, was found to be progressively deteriorating, so that the great majority of experiments were carried out on the second sample (chlorophyll Z.Z.) which contained a small percentage of copper, added as a stabiliser. Approximately 5 gms. of crude paste were shaken in the dark for 12 hours with 500 mls. of A.R. acetone. The resultant solution was filtered and stored in the dark. It contained both chlorophyllic and carotenoid molecules. To obtain a chlorophyllic fraction, the following procedure was carried out. Part of the stock solution (30 - 50 mls.) was evaporated to dryness in a small conical flask. The resultant green-black film was taken up as a slurry in ~30 mls of petroleum ether (60-80), and transferred to the top of a bone-meal chromatograph column. The chlorophylls are only slightly soluble in petroleum ether but the carotenoid molecules were slowly extracted by continuous washing with petroleum ether and passed down the column. Such chlorophyll as did dissolve in the petroleum ether was strongly adsorbed as a dark green band at the top of the column. After thorough washing, all carotenes were removed from the sample. The chlorophyll fraction was then eluted with acetone, filtered, made up to 100 mls. and stored in the dark. The bone-meal column used was approximately 10 cms. long and 2 cms. in diameter. It served also to remove a green impurity, probably a

degradation product which was retained at the top of the column. The chromatographic purification was occasionally repeated on the chlorophyll solution and a pale green band invariably appeared at the top of the column, supporting the hypothesis of a degradation product. The column was prepared from bone-meal which had been extracted in a Soxhlet apparatus with alcohol, for 12 hours, and with acetone for the same period. After extraction it was ground to pass a 100 mesh sieve. The column was formed by the addition of a slurry of bone-meal in 60-80° petroleum ether to a chromatograph tube, the resultant column being thoroughly washed with petroleum ether before use.

: Concentration of pigment solutions.

The concentrations of the solutions were found by evaporation of measured volumes to dryness in weighed crystallising dishes, placed in a vacuum desiccator. Determinations were usually repeated in triplicate.

: Absorption spectra.

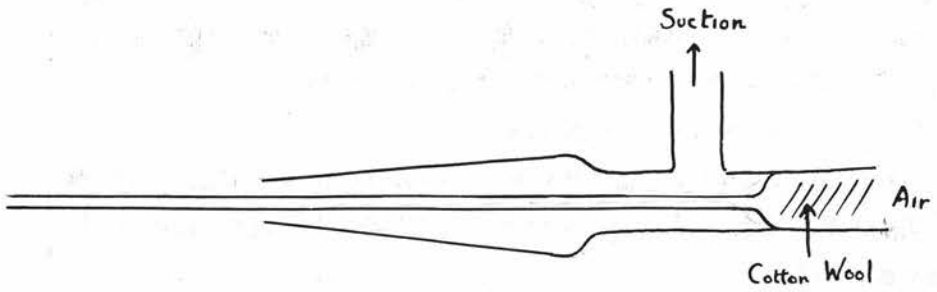
Absorption spectra were taken using a "Unicam" (S.P.500) spectrometer, and were used to check on possible degradation of solutions and also, after a preliminary calibration, as an alternative method of finding concentrations of solutions. Values of $\log I^{\circ}/I$ were measured over a spectral range of 400 - 800 mμ, readings being taken every 10 mμ over the entire range and every 5 mμ in the region of the absorption maxima.

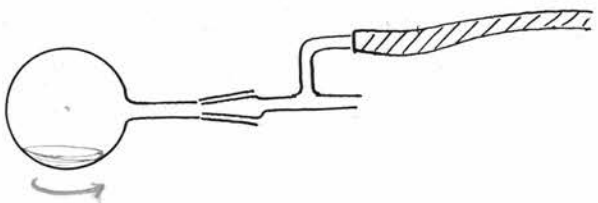
: Carotene solutions.

The petroleum ether washings from the chromatograph column were evaporated to dryness, redissolved in acetone and the concentration found by a similar procedure.

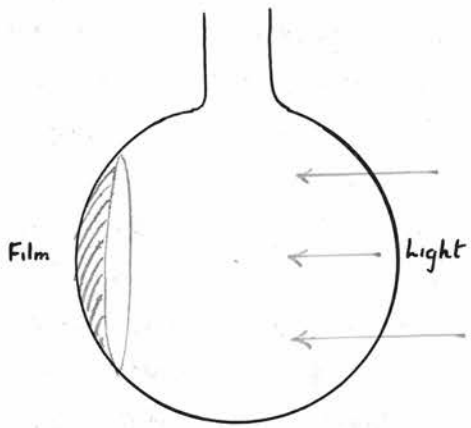
: Anhydrous oxalic acid solution.

Crystals of oxalic acid hydrate, $(CO_2H)_2 .2H_2O$, were heated for several hours at 100°C. until complete dehydration had occurred. This was confirmed by finding the equivalent weight by permanganate titration. A small quantity of the anhydrous





Circular movement to form "even" film



crystals was dissolved in anhydrous acetone, (A.R. acetone dehydrated by calcium chloride) and the concentration of the solution found by permanganate titration.

: Thallous bromide substrate.

Throughout most of the work thallous bromide was used as a substrate and as a photo-sensitiser. It was prepared by the slow addition of thallous nitrate solution to potassium bromide solution. The amounts generally used were 100 mls. of thallous nitrate solution containing 5.32 gms. of thallous nitrate, and 50 mls. of potassium bromide solution containing 2.50 gms. of potassium bromide. Under such conditions, precipitation always occurred in the presence of an excess of bromide ions. The resultant precipitate was washed several times with distilled water, by decantation, dried over phosphoric oxide in a vacuum desiccator, and stored in the dark.

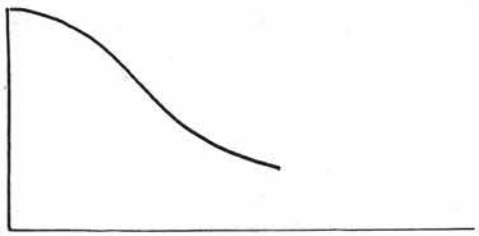
: Preparation of the film and general procedure.

A weighed quantity of thallous bromide, normally 0.3 gm., was introduced into the reaction vessel, followed by a measured volume of pigment solution. The resultant slurry was evacuated to dryness, using the arrangement shown. During evacuation the reaction vessel was rotated in such a manner as to produce a smooth film on one side. The reaction vessel was then placed in the apparatus and evacuated for several hours (minimum 2 hours), allowed to stand overnight and then "rinsed out" with ~20 mm. of oxygen (1 hour's pumping). The thermostating system was started during the rinsing out process. Oxygen and other gases were admitted to the desired pressures. After a short period the gauge taps were closed, and if no movement of the gauge pointer occurred inside half an hour, illumination of the film was started. This was the general procedure. It has been thought better to discuss specific procedures with the experiments involved, where the procedure is highly dependent on the nature of the experiment.

Experimental results.

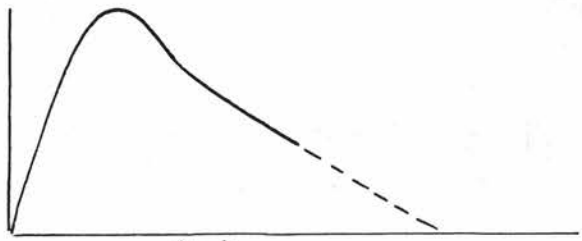
A characteristic of the kinetics of the photo-degradation of chlorophyll is the fact that the plot of rate of reaction, measured as rate of pressure change, against total reaction, measured as total pressure change, becomes linear shortly after the commencement of the reaction, and remains linear up to 90% total reaction(23).

Pressure decrease or overall reaction (Δp)



Time

Rate of pressure decrease $\frac{\partial}{\partial t}(\Delta p)$



(Δp)

It is thus possible to estimate the total amount of reaction by extrapolation to zero rate. Knowing the gauge sensitivity and the volume of the reaction space it is possible to correlate the total pressure change in the gas phase with the amount of pigment present and exposed to illumination. The ratio : moles of pressure decrease / moles of pigment : will be referred to as ϕ throughout.

If $\frac{\partial}{\partial t}(\Delta p)$ decreases linearly with Δp

then $\frac{\partial}{\partial(\Delta p)}\left\{\frac{\partial(\Delta p)}{\partial t}\right\} = K$

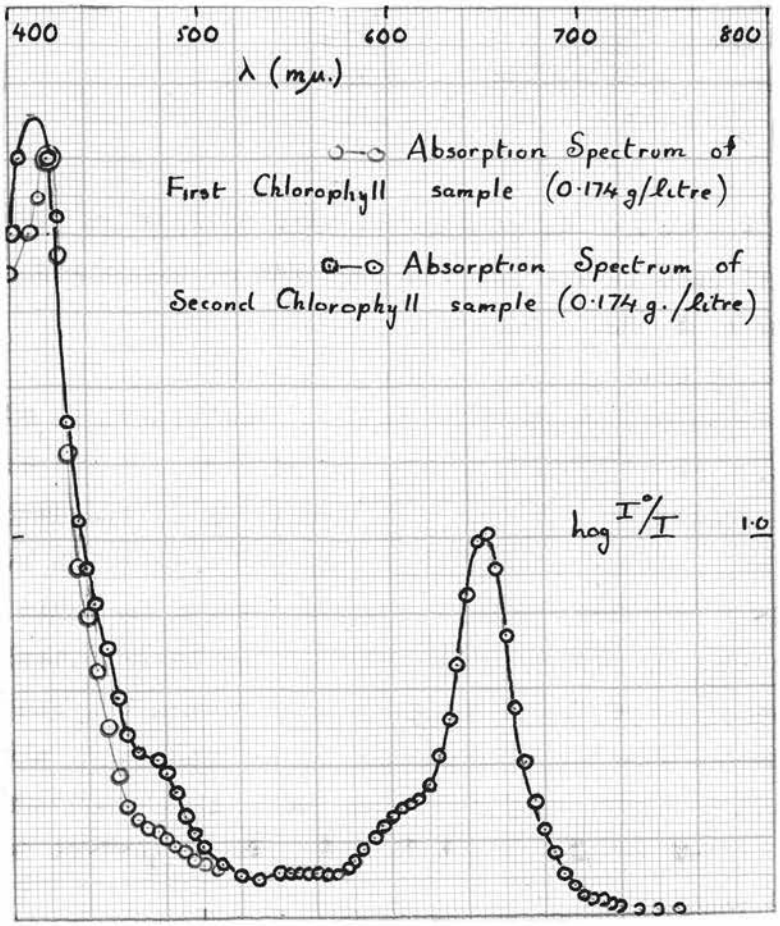
therefore $\frac{\partial(\Delta p)}{\partial t} = K\Delta p + \text{const.}$

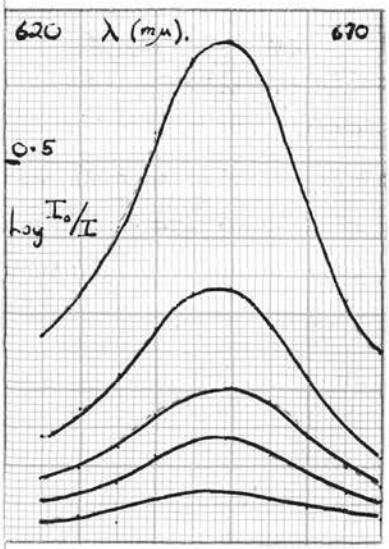
at $t = \infty \quad \Delta p = \Delta p_\infty$ and $\frac{\partial(\Delta p)}{\partial t} = 0$

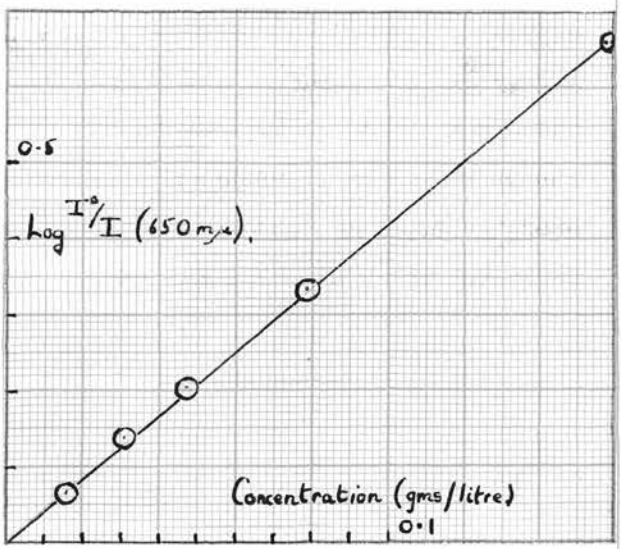
therefore $\text{const.} = -K\Delta p_\infty$

therefore $\frac{\partial}{\partial t}\Delta p = K(\Delta p - \Delta p_\infty)$

i.e $\frac{d.\Delta p}{(\Delta p_\infty - \Delta p)} = -K\,dt$ or $-\log(\Delta p_\infty - \Delta p) = -Kt + \text{const.}$


400
500
600
700
800
λ (mμ.)
Absorption Spectrum of First Chlorophyll sample (0·174 g/litre)
Absorption Spectrum of Second Chlorophyll sample (0·174 g./litre)
log I°/I
1·0


620
λ (mμ).
670
0·5
log I₀/I


0·5
log I°/I (650 mμ).
Concentration (gms/litre)
0·1

At zero time $\Delta p = 0$ therefore $const = -\log \Delta p_\infty$

therefore $\log(\Delta p_\infty - \Delta p)/\Delta p_\infty = kt$

i.e. $(\Delta p_\infty - \Delta p)/\Delta p_\infty = e^{kt}$

or $\Delta p = \Delta p_\infty (1 - e^{kt})$

This expression is similar to that derived on the basis of the law of mass action for the sequence of unimolecular reactions $A \rightarrow B \rightarrow C$.

: Absorption spectra of pigment solutions.

The first diagram shows the absorption spectrum of the first chlorophyll sample, suspected of degradation, compared with the absorption spectrum of the second intact sample. The two spectra are identical in the red absorption band, which would indicate that the degradation is not akin to phaeophitinisation which, although giving an increased value for $\varnothing$, would in addition alter the red absorption band. in addition the blue absorption band is not radically altered, so that in all probability the central magnesium chlorin nucleus of the chlorophyll structure remains intact in the degraded sample.

The plot of log I°/I against concentration was found to be linear at low concentrations, which enabled a check to be made on the concentrations of the pigment solutions used. The effect of thallous bromide on the absorption spectrum of the chlorophyll solution was found to be almost negligible; the essential form of the curve remains unaltered, the peaks being slightly diminished in height, presumably due to residual adsorption, for thallous bromide treated with a chlorophyll solution retains a slight green colouration.

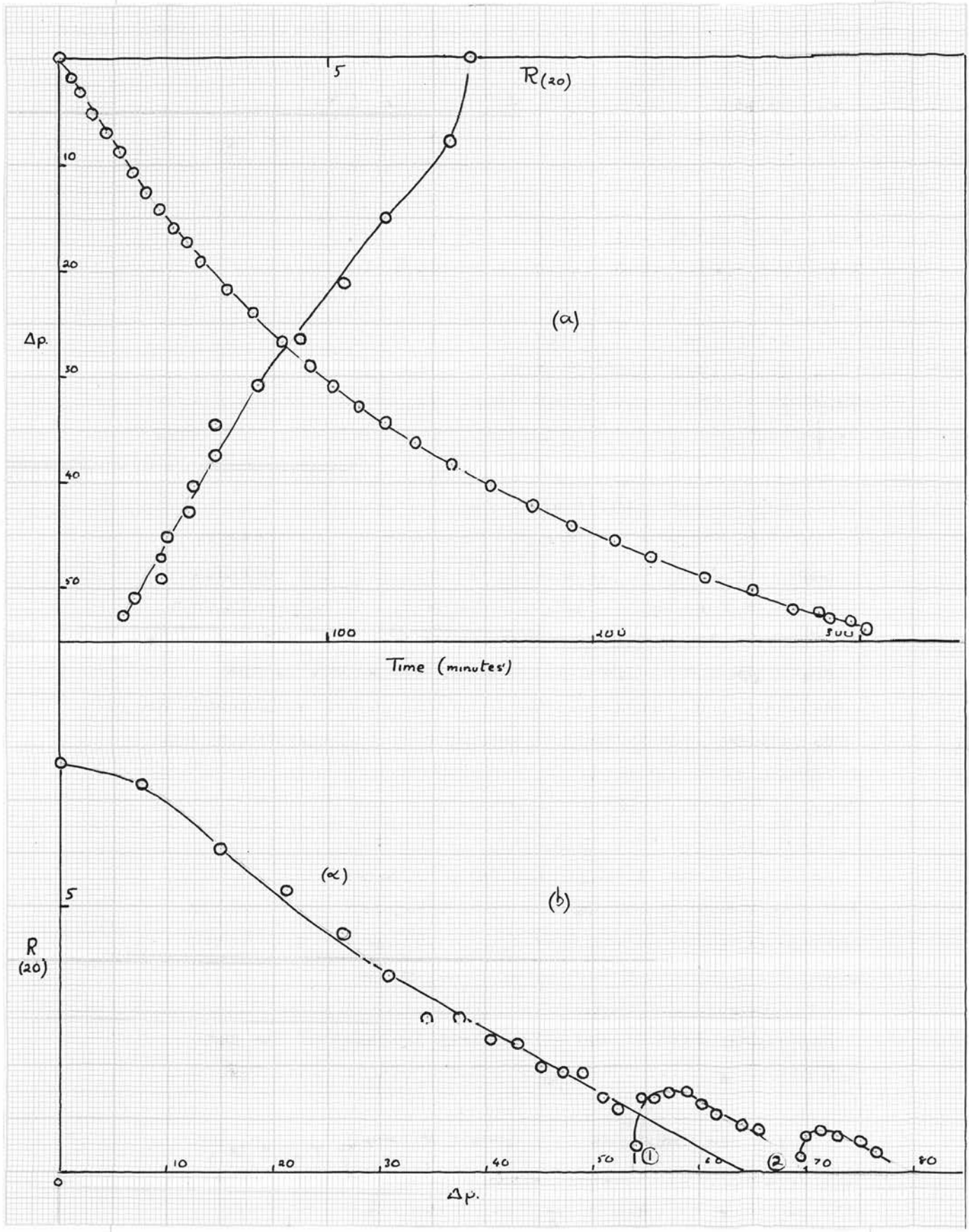
Calibration curve

Conc.	0.157	0.0785	0.0471	0.0314	0.0157	(gms./L.)
λ			Log. I°/I.			
625	0.270	0.139	0.083	0.052	0.026	
630	0.325	0.173	0.099	0.065	0.036	
635	0.412	0.220	0.126	0.078	0.045	
640	0.538	0.280	0.166	0.111	0.057	
645	0.634	0.329	0.195	0.133	0.065	
650	0.660	0.331	0.201	0.139	0.067	
655	0.589	0.280	0.183	0.115	0.056	
660	0.448	0.218	0.138	0.093	0.045	
665	0.318	-	0.100	0.065	0.042	
670	0.250	0.110	0.073	0.052	0.030	

λ , the wavelength in mμ.

: Photo-oxidation of the first chlorophyll sample at high oxygen pressure.

The purpose of this experiment was to ascertain whether degradation of this sample had occurred by a check on the value of ϕ and also on the"reversibility"effect on evacuation. The normal pressure decrease occurred on illumination and the pressure decrease / time curve is shown at (a). The rate curves derived from this and other similar curves for illumination periods separated by periods of evacuation, are shown at (b). The same film was used throughout. At (1) and (2) the system was evacuated and left overnight before re-admission of oxygen and re-illumination. It will be noted that an increased rate of reaction was found following the periods of evacuation. The extrapolated value of ϕ was 1.38. From this result it is evident that the first sample of chlorophyll, though still showing the "reversibility" effect, had suffered some form of degradation, since previous work on the chlorophyll fraction of this sample had given a value of unity for ϕ. Work was accordingly discontinued on this sample.


5
R(20)
10
20
Δp.
30
40
50
(a)
100
200
300
Time (minutes)
(c)
(b)
5
R
(20)
0
10
20
30
40
50
60
70
80
Δp.
①
②

0.3gm. TlBr. 0.00280gm. Chlorophyll.
94mm. Oxygen. Temperature 25°C.
Reaction volume 58.0mls. Gauge Sensitivity 0.00216mm. /0·1 scale division.

Δp	R	Δp	R	Δp	R	Δp	R
0.0	7.7	42.9	2.4	55.8	1.4	-	-
7.7	7.3	45.1	2.0	57.2	1.5	69.6	0.3
15.0	6.1	47.1	1.9	58.7	1.5	69.9	0.7
21.1	5.3	49.0	1.9	60.2	1.3	71.3	0.8
26.4	4.5	50.9	1.4	61.5	1.1	72.8	0.7
30.9	3.7	52.3	1.2	62.6	1.2	74.9	0.6
34.6	2.9	-	-	63.8	0.9	76.4	0.4
37.5	2.9	53.9	0.5	64.7	0.9		
40.4	2.5	54.4	1.4	65.6	0.8		

R, the rate of pressure change, was taken as the pressure change occurring in 20 minutes, as read from the pressure/time curve. The approximation involved is the approximation of chord to tangent on the pressure change (or pressure) /time curve. This method was thought to be preferable to the construction of tangents over the small ranges considered. The time interval was varied, as convenient, in measuring rates in different experiments.

Extrapolated value of $\Delta p = 64$ scale divisions.

$\equiv 64 \times 0.0216 \times 58 \times (273/298) \times (1/22{,}400)$

$= 4.31 \times 10^{-6}$ moles of gas pressure decrease.

Taking a value of 900 for the mean molecular weight of the mixture of chlorophylls α and β, the amount of chlorophyll present $= 2.80 \times 10^{-3}/900 = 3.1 \times 10^{-6}$ gm. moles.

therefore $\phi = 1.38$.

: Photo-oxidation of the chlorophyll fraction of the second commercial sample. (Chlorophyll Z.Z., oil soluble, McFarlan and Co.)

After the formation of a normal chlorophyll film, the system was evacuated for 8 hours, left overnight, rinsed out with oxygen and 99mm. of oxygen added. After the normal

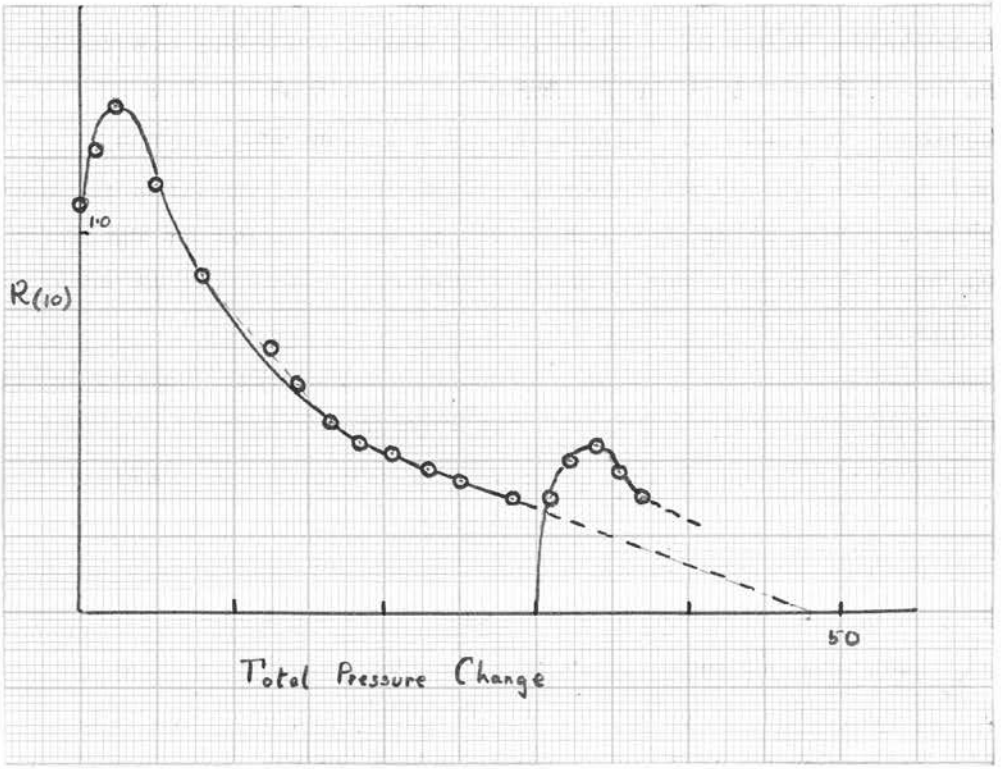

1.0
R(10)
50
Total Pressure Change

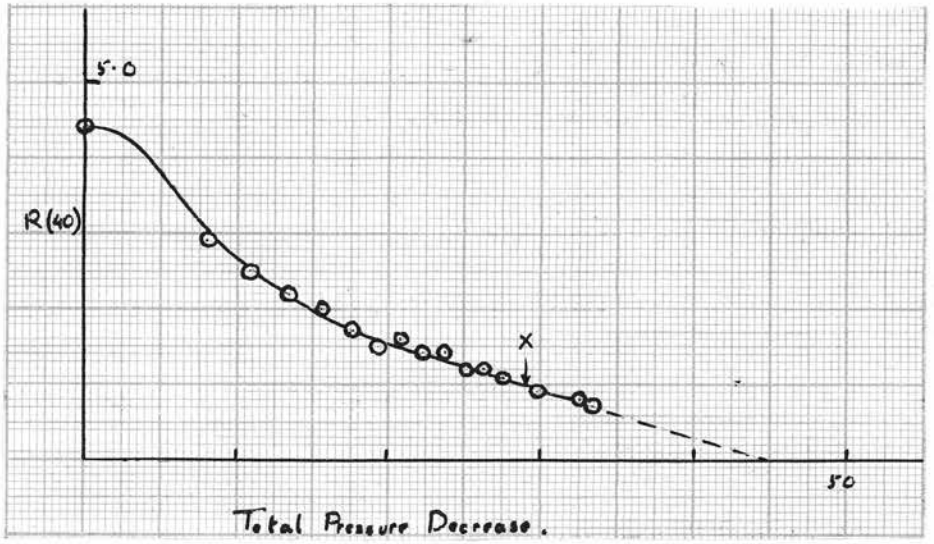
photo-oxidation had been carried out for the uptake of 31 scale divisions, the system was evacuated and left overnight. On re-introduction of oxygen and re-illumination, increased rates were obtained indicating that the new sample exhibited the "reversibility" effect shown by its predecessor. The extrapolated value of ϕ was 0.99, showing that the new sample gave an equivalent pressure decrease to the old prior to deterioration. In both this and the previous experiment, emphasis was placed on obtaining a good extrapolation, so that the"reversibility"effect was by no means as great as would have been obtained, had evacuation been carried out at an earlier point.

0.3gm. TlBr. 3.78 x 10^{-6} gm. moles Chlorophyll.
99mm. Oxygen Temperature 25°C.
Reaction volume 69.3mls. Gauge sensitivity 0.0210mm./ scale division.

Δp	R(10)	Δp	R	Δp	R	Δp	R
0.0	1.08	12.3	0.70	22.9	0.38	32.2	0.40
1.1	1.22	14.3	0.60	25.2	0.35	33.8	0.41
2.3	1.34	16.5	0.50	28.5	0.30	35.5	0.37
4.9	1.13	18.4	0.45	-	-	37.0	0.30
8.0	0.89	20.7	0.42	31.0	0.30		

Δp_∞ = 48.0 scale divisions
$\equiv$ (69.3 x 0.0210 x 48 x 273/22,400 x 298 x 760)gm. moles
= 3.75 x 10^{-6} gm.moles
therefore ϕ = 0.99.

Since, as has been mentioned in the introduction, the extrapolated value of ϕ is markedly altered by the presence of absorbents such as phosphoric oxide, an attempt was made to get consistent values of ϕ for oxidations carried out under these conditions. It was again found that a linear extrapolation was feasible but it did not prove possible to obtain reproducible results.


5·0
R(40)
X
50
Total Pressure Decrease.

: Photo-oxidation of a chlorophyll film in the presence of phosphoric oxide.

0.3 gm. TlBr. 5.7×10^{-7} gm mole chlorophyll.

99mm. oxygen Temperature 25° C.

Reaction volume 59.9 mls. Gauge sensitivity 0.0209 mm./ scale division

Phosphoric oxide in side tube.

Δp	R(40)	Δp	R	Δp	R	Δp	R
0.0	4.4	15.6	2.0	22.4	1.4	27.6	1.1
8.0	2.9	17.6	1.7	23.8	1.4	29.8	0.9
10.9	2.5	19.3	1.5	25.2	1.2	32.6	0.8
13.4	2.2	20.8	1.6	26.4	1.2	33.4	0.7

Δp_∞ = 44.6 scale divisions

≡ (44.6 x 0.0209 x 59.9 x 273/760 x 22,400 x 298) gm. moles of pressure decrease.

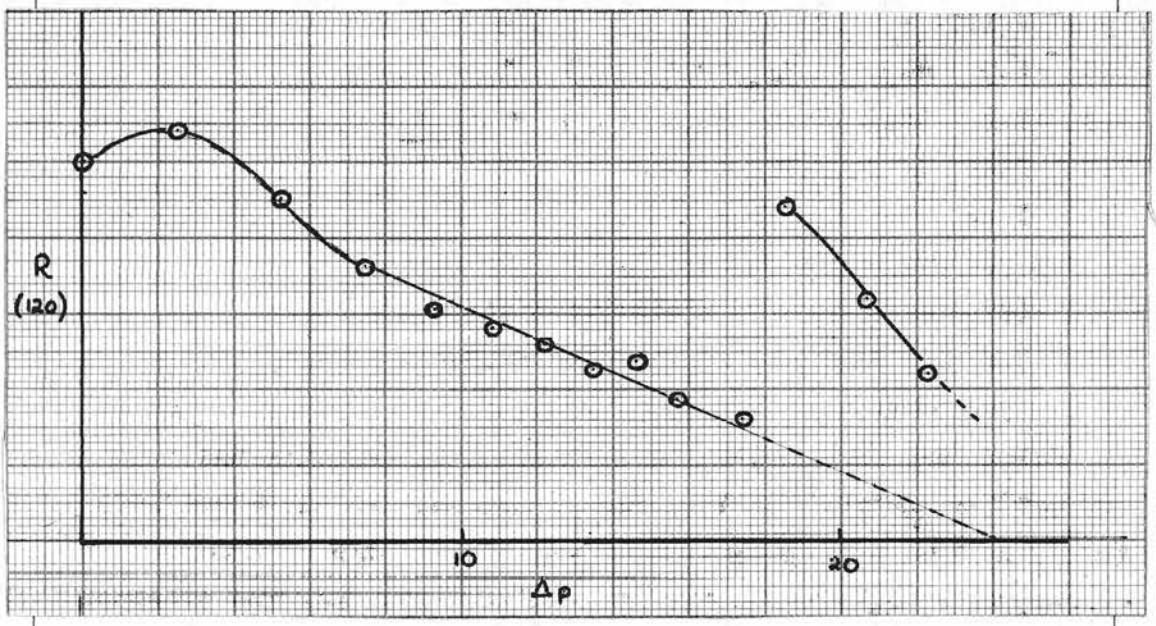
= 30.1×10^{-7} gm. mole

∴ $\phi = 30.1/5.7 = 5.3$

At the poimt X, illumination was discontinued and the apparatus left running overnight. The film was left standing in the presence of oxygen and the gauge taps were closed. It is obvious that no "reversibility" effect occurs in the presence of phosphoric oxide, with the proviso that the gas phase is not removed. A small diffusion effect ~ 1 scale division of additional pressure decrease, was found overnight, presumably due to the slow uptake of some reaction product by the phosphoric oxide.

Other values of ϕ obtained with the same reaction vessel, under the same conditions were 4.6 and 2.7. Fresh phosphoric oxide was used in each case.

Using a reaction vessel of slightly different shape and greater volume, the previous experiment was repeated. The values obtained for ϕ were considerably less.


R
(120)
10
20
Δp

0.3 gm. TlBr. | 1.75×10^{-6} gm. mole chlorophyll.

100mm. oxygen. | Temperature 25° C.

Reaction volume 83.5mls. | Gauge sensitivity 0.0330 mm./ scale division.

Phosphoric oxide in side tube.

Δp	R(120)	Δp	R	Δp	R	Δp	R
0.0	2.5	9.3	1.53	14.6	1.18	18.5	2.2
2.5	2.7	10.8	1.40	15.7	0.93	20.7	1.6
5.2	2.25	12.2	1.29	17.4	0.84	22.3	1.1
7.5	1.82	13.5	1.13	-	-	-	-

Δp_{∞} = 24 scale divisions

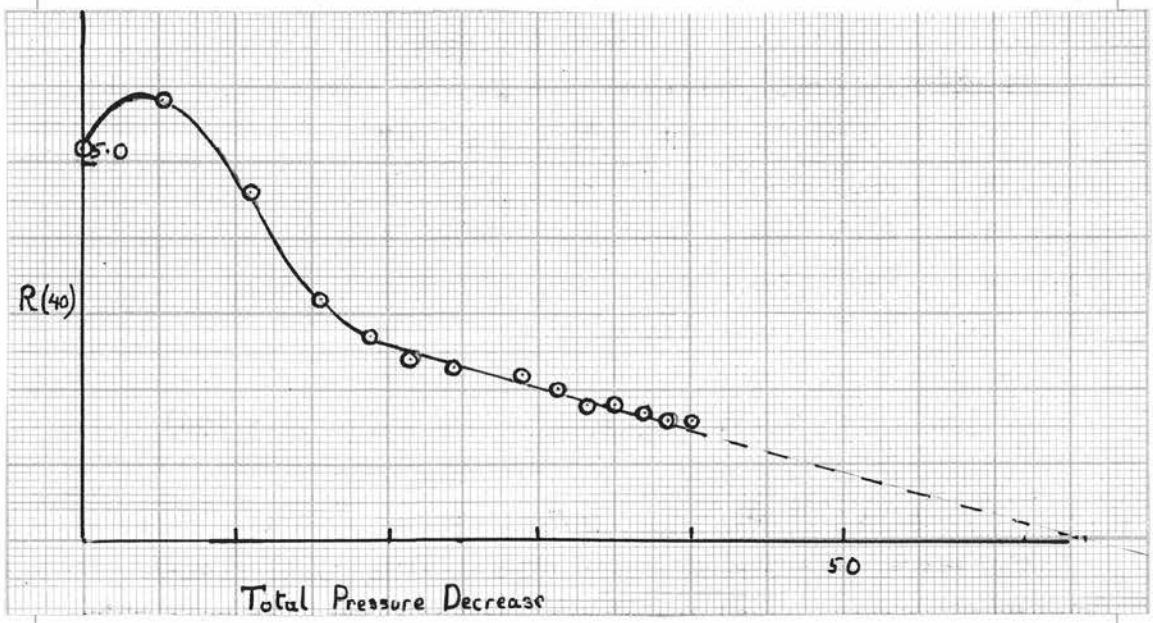
= (83.5 x 24 x 0.033 x 273/760 x 298 x 22,400)

= 3.6×10^{-6} gm. mole of pressure decrease.

therefore ϕ = 3.6/1.75 = 2.05.

After the first period of illumination, on which the extrapolated value is based, the system was evacuated and left for 72 hours. On re-illumination in the presence of 100mm. of oxygen, a marked increase in rate was obtained. The presence of phosphoric oxide had previously been reported to destroy the "reversibility" effect.(25). It is important to note that the "non-reversibility" corresponds to high values of ϕ. A repeat of the experiment gave a value of 1.75 for ϕ.

It would seem evident from these results that a gaseous product is involved in the reaction, and it may be pointed out here that the non-reproducibility of ϕ could be due to to complications arising out of diffusion and dissociation effects. There remained the possibility, however, that the effect of phosphoric oxide was a pre-treatment effect, akin to extensive dehydration. Against this point of view, it had been found (26) that even evacuation to 10^{-6} mm. did not appreciably alter the "normal" kinetics.


5.0
R(40)
50
Total Pressure Decrease

: Photo-oxidation of chlorophyll in the presence of phosphoric oxide and soda-lime.

0.3 gm TlBr. 5.7×10^{-7} gm. mole chlorophyll
100 mm. oxygen Temperature 25° C.
Reaction volume 59.9mls. Gauge sensitivity 0.0209 mm./ scale division.

Phosphoric oxide and soda-lime in side tube.

Δp	R(40)	Δp	R	Δp	R	Δp	R
0.0	5.2	18.8	2.7	29.0	2.2	36.8	1.7
5.2	5.8	21.5	2.4	31.2	2.0	38.5	1.6
11.0	4.6	24.4	2.3	33.2	1.8	40.1	1.6
15.6	3.2	26.7	2.3	35.0	1.8	-	-

$\Delta p_{\infty} = 66$

$\equiv (66.0 \times 0.0209 \times 59.9 \times 273 / 760 \times 22{,}400 \times 298)$

$= 44.2 \times 10^{-7}$ gm. mole of pressure decrease

therefore $\phi = 44.2/5.7 = 7.8$.

The reaction appeared to be slightly faster than with phosphoric oxide alone. The value obtained for ϕ is well above the value obtained with phosphoric oxide alone, but is not consistent with the maximum value of 6, previously reported for a similar experiment (27). Assuming that ϕ is increased to a value of 6 or above in contrast with values of 5 in the presence of phosphoric oxide alone, it would appear that a second, acid gas is involved in the reaction. Some evidence for carbon dioxide formation had been previously found on treating condensables from the gas phase with Baryta solution (28). and in the micro-distillation experiments to be described later, evidence of another gas besides water vapour was again found.

: Summary of Absorption experiments.

In the presence of phosphoric oxide, the value of ϕ varies between 2 and 5. It seems possible that diffusion and dissociation effects can cause this lack of reproducibility. With soda-lime, ϕ rises still further to a value of 6 or possibly 8. The participation, in the photo-oxidation, of two gases besides oxygen, seems indicated.

A possible reaction scheme :

$$Chl + nO_2 \rightarrow ChlO_2 + A \quad (1)$$

$$A + mO_2 \rightarrow (m+n-1)B \quad (2)$$

was adopted as a basis for future work. Chl is unreacted chlorophyll, $ChlO_2$, an oxidised product of chlorophyll and A and B are gaseous reaction products. This reaction scheme has a certain attraction in that, assuming (1) and (2) to be unimolecular, it explains the linear decrease of rate with total reaction and the "reversibility" effect. It was later shown to be false in many respects, e.g. it gives no explanation of dissociation pressure effects.

: Experiments involving the use of the three-way tap reaction vessel in attempts to obtain further information on the reaction mechamism.

: (a) Proof that the effect of absorbents is a "real" and not a "pre-treatment" effect.

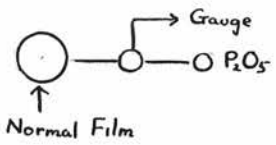
On the basis of the previous experiments there exists the possibility that the increased value of ϕ is due to some reaction sequence such as :

$$Chl \rightleftharpoons A(solid) + X(gas) \quad (1)$$

$$X + P_2O_5 \rightarrow (P_2O_5X) \quad (2)$$

$$A + 5O_2 \xrightarrow{h\nu} A(5O_2) \quad (3)$$

Chlorophyll may be in equilibrium with a solid A and a gas X (1). X may be "taken up" rapidly by phosphoric oxide (2). A may react photo-chemically with 5 molecules of oxygen in consecutive reactions (3). Varying efficiency of process (2) would give values of from one to five for ϕ.



The arrangement used was a shown. A spherical reaction vessel containing a normal film, was attached to one side of the three-way tap, a small tube containing phosphoric oxide to the

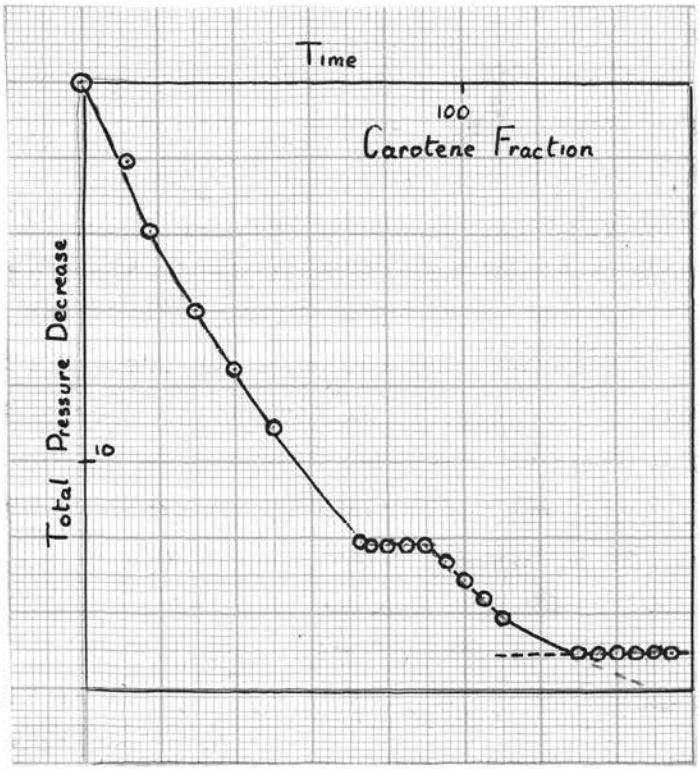
100
150
200
250
10
20
Pressure Decrease
← Light Off.
← P_2O_5 In.
← P_2O_5 Out.
← Light Off.
← P_2O_5 In.
← P_2O_5 Out.
Chlorophyll.

other. The system was evacuated for 13 hours, left standing for 12 hours, rinsed out with 20 mm. of oxygen, filled with oxygen to a pressure of 100mm. and the "normal" reaction carried out with the tap first turned to position ⊕ to exclude phosphoric oxide. On cessation of illumination and connecting in phosphoric oxide an additional pressure decrease took place which ceased on again excluding the phosphoric oxide. The "normal" pressure decrease, in this case, was found to be practically the same as that obtained with a similar film initially evacuated in the absence of phosphoric oxide. The slight "jump" on connecting in the phosphoric oxide is due to, initially, the existence of a slight inequality of pressure between reaction vessel and side tube.

0.3 gm. TlBr. 13.6 x 10^{-6} gm. mole chlorophyll
100mm. oxygen. Temperature 25° C.
Reaction volume 54.1mls. Gauge sensitivity 0.0209mm./scale division.

Time	Δp	Time	Δp	Time	Δp	Time	Δp
0	0	115	11.4	205	19.0	0	0
10	1.0	125	12.0	210	20.0	15	1.0
20	2.8	150	13.7	215	21.0	30	3.0
30	4.0	160	14.0	220 Z	22.0	55	6.0
35	4.3	170 X	14.4	225	22.0	65	7.0
65	7.2	185	15.0	230	22.0	80	9.0
75	8.0	175	14.8	-	-	93	10.0
85	9.0	190 Y	15.0-14.0		-	105	11.0
95	10.0	195	16.0				
105	10.9	200	18.0				

Times are in minutes, pressure changes in scale divisions. The fourth column gives the normal reaction in the absence of phosphoric oxide , all other conditions being identical. At X illumination was discontinued, at Y phosphoric oxide was connected in, and at Z phosphoric oxide was again excluded. The part of the relevant graph enclosed in the rectangle is shown on an enlarged scale for clarity.


Time
100
Carotene Fraction
Total Pressure Decrease
10

: (b) Demonstration of a similar effect with carotenes.

The conditions and procedure were identical except that 0.00225 gm. carotene was used instead of chlorophyll.

Time	Δp	Time	Δp	Time	Δp	Time	Δp
0	0	50	9.1	90 Y	12.2	130 Z	15.1
12	2.1	73	12.1	95	12.6	135	15.1
18	3.9	75 X	12.2	100	13.1	140	15.1
30	6.0	80	12.2	105	13.6	145	15.1
40	7.5	85	12.2	110	14.1	155	15.1

The points X, Y and Z have the same significance as before.

It would seem quite definite from these experiments that the effect of phosphoric oxide is a "real" effect, not a "pre-treatment" effect and that at least one gaseous product other than oxygen and capable of absorption by phosphoric oxide, is involved in the reaction.

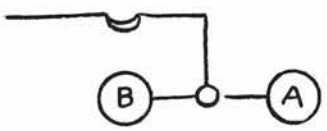
: (c) Attempt to separate the reaction into separate stages.

If we accept as a basis, the reaction scheme :

$$Chl + nO_2 \rightarrow ChlO_2 + A(gas) \qquad (1)$$

$$A + mO_2 \rightarrow (m + n - 1)B(gas) \qquad (2)$$

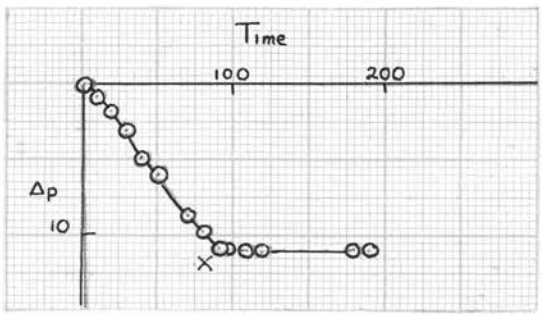
: then it is obvious that reaction (2) is a pressure increase reaction. If, in addition, it were possible to separate reactant A from contact with the chlorophyll film, then reaction (2) should be demonstrable as a pressure increase reaction.



The double reaction vessel shown was used.

Reaction volumes : A = 35.7mls.
B = 35.9mls.
A + B + Gauge = 90.omls.

In reaction vessel A was placed a film of 3.42×10^{-6} mole of chlorophyll on 0.3gm. TlBr. In reaction vessel B, a film of thallous bromide only was placed. With the tap in position ⊖ both films were thoroughly evacuated for 3 hours, left standing overnight and "rinsed out" with 20mm. of oxygen.


Time
100
200
Δp
10

The tap was then turned ⊕ and 100mm. of oxygen admitted to bulb A. The normal reaction was then carried out for a pressure decrease of ~10 scale divisions. The tap was then changed to position ⊖, so that the gas phase over the chlorophyll film was partly transferred to the evacuated bulb B. The gauge taps were then opened and the pressure in the gauge jacket and gauge reduced to 50mm. to prevent damage to the gauge on connecting in bulb B. The tap was then turned ⊕, to connect bulb B to the gauge, also excluding the chlorophyll film. The gauge taps were then closed and the system allowed to reach equilibrium. On illumination of bulb B no pressure decrease or increase was observed.

Time	Δp	Time	Δp	Time	Δp	Time	Δp
0	0.0	40	4.9	90	10.9	120	0.0
10	0.9	50	6.0	- X	-	125	0.0
20	1.9	70	8.7	95	0.0	180	0.0
30	3.1	80	9.8	110	0.0	190	0.0

Up to the point X the readings give the normal reaction as carried out in bulb A. From X onwards the readings correspond to the illumination of bulb B.

Although these results exclude reaction (2) from being a photo-chemical reaction sensitised by thallous bromide, there remains the possibility that it is sensitised by chlorophyll itself.

: (d) Attempt to demonstrate the existence of consecutive recations assuming the second to be chlorophyll-sensitised.

Exactly the same procedure was use in this experiment as in the previous one, except that both reaction vessels contained a thallous bromide/chlorophyll film. It was hoped by the transfer of some of the gas phase to an unoxidised film, to upset the equilibrium sufficiently to give a marked pressure increase. No such effect was found, only the normal pressure decrease being observed in both cases.

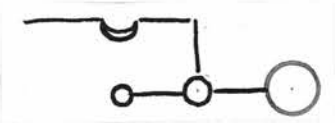
Time	Δp	Time	Δp	Time	Δp	Time	Δp
0	0.0	62	8.0	137	15.1	170	17.3
15	1.9	67	9.0	147	16.0	185 Y	17.3
25	3.0	75	9.8	150	16.1	195	18.0
35	4.9	105	13.0	160 X	17.0	210	19.0
45	6.0	120	14.0	-	-	228	20.0

At X the transfer was carried out. At Y bulb B was illuminated after the system had come to equilibrium.

Both attempts to demonstrate the existence of a pressure increase reaction directly had been unsuccessful.

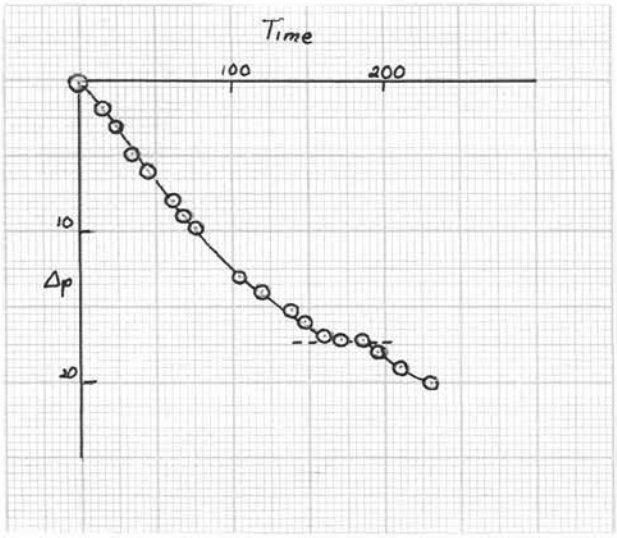
: (e) The effect of phosphoric oxide on the rate of the "normal" reaction.

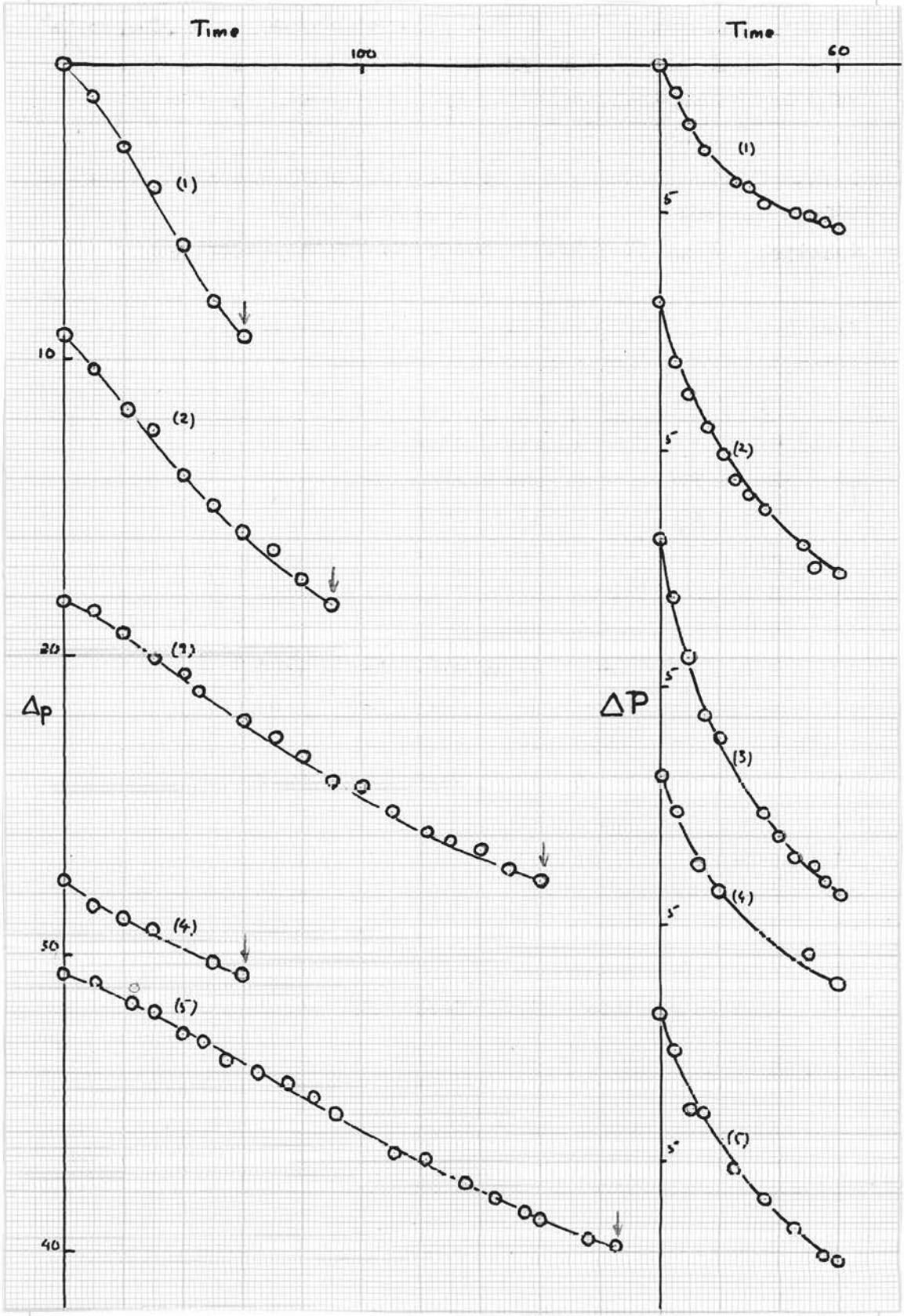
Before any attempt was made to determine the actual gas or gases present, the effect of exposing the gas phase to phosphoric oxide between successive periods of illumination was examined. It was initially hoped to correlate the normal pressure decrease with the amount of gaseous product formed during the reaction.



P_2O_5 Normal film

The arrangement used was as shown above. A film was evacuated in the normal manner and oxygen admitted to the system, to a pressure of 100mm. Before commencing illumination, the phosphoric oxide was excluded by turning the tap ⊕. At the points marked ↓ on the graph, the light was switched off nad the tap turned ⊖. The amount of gaseous product taken up by the phosphoric oxide in one hour was measured, the phosphoric oxide again excluded and the procedure repeated. After the fourth repetition the film was allowed to stamd overnight in 100mm. of oxygen, phosphoric oxide being present. The procedure was again repeated the following day. On the assumption that the volume of the side tube was negligible


Time
100
200
10
Δp
20

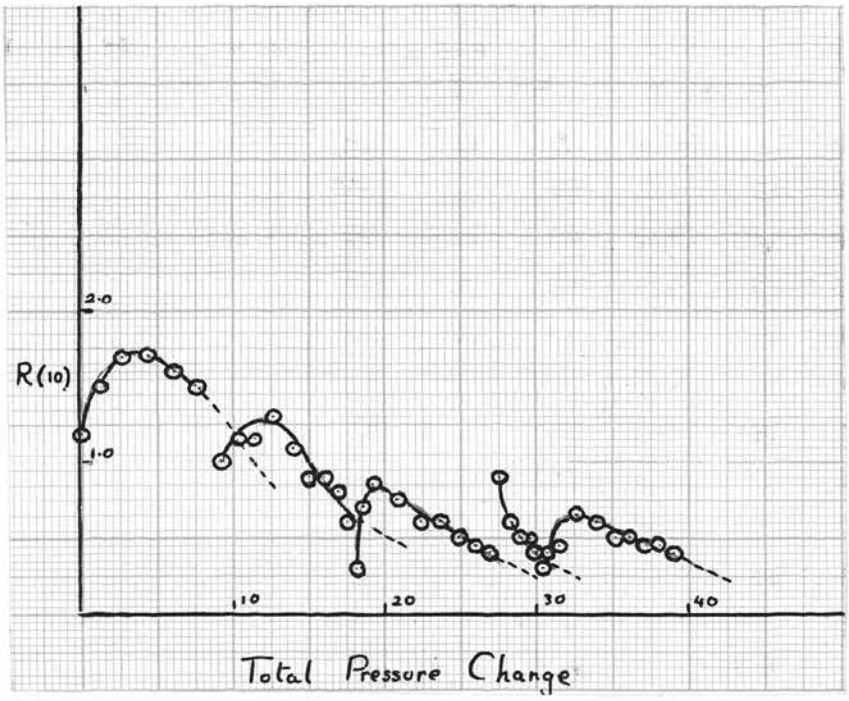

Time
100
(1)
10
(2)
20
(3)
Δp
(4)
50
(5)
40
Time
60
(1)
5
(2)
5
5
ΔP
(3)
(4)
5
(5)
5

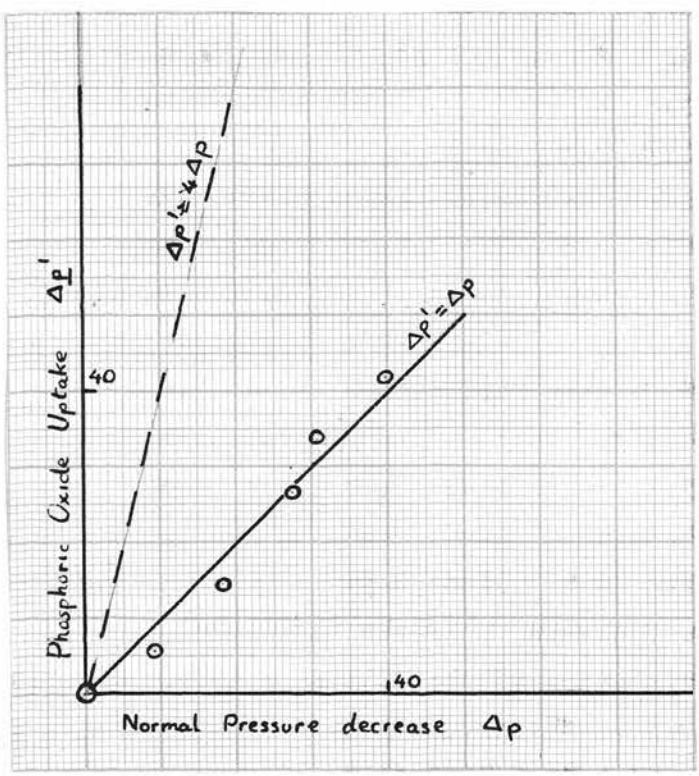
in comparison with that of the reaction vessel, the phosphoric oxide uptake was plotted against the normal pressure decrease. Rate curves were also plotted for the various periods of normal reaction.

0.3gm. TlBr. 5.2×10^{-6} mole chlorophyll.
100mm. oxygen. Temperature 25°C.
Reaction volume 54.1mls. Gauge sensitivity 0.0210mm. / scale division.

Time t. (mins.) Total normal pressure decrease Δp (scale divisions)

t	Δp	t	Δp	t	Δp	t	Δp
0 (1)	0.0	80	17.4	122	25.9	30	32.0
10	1.1	90	18.2	130	26.2	40	32.7
20	2.8	91	18.2	140	26.5	47	33.0
30	4.2	-	-	150	27.2	55	33.6
40	6.1	0 (3)	18.2	160	27.5	65	34.0
50	8.0	10	18.5	-	-	75	34.4
60	9.2	20	19.3	0.(4)	27.5	84	34.8
-	-	30	20.1	10	28.4	91	35.4
0 (2)	9.2	40	20.6	20	28.8	111	36.7
10	10.3	45	21.2	30	29.2	122	36.9
21	11.7	60	22.2	50	30.3	135	37.7
30	12.4	71	22.7	60	30.7	145	38.2
40	13.9	80	23.4	-	-	155	38.7
50	14.9	90	24.2	0 (5)	30.7	160	38.9
60	15.8	100	24.4	11	31.0	176	39.6
70	16.4	110	25.2	23	31.7	185	39.8


R(10)
2.0
1.0
10
20
30
40
Total Pressure Change


Phosphoric Oxide Uptake Δp'
40
Δp'=½Δp
Δp'=Δp
40
Normal Pressure decrease Δp

Time t. (mins.) Phosphoric oxide uptake ΔP (scale divisions)

t	ΔP	t	ΔP	t	ΔP	t	ΔP
0 (1)	0.0	5	2.0	10	4.0	20	3.9
5	1.0	10	3.1	15	5.9	50	6.0
10	2.0	16	4.2	20	6.7	60	7.0
15	2.9	21	5.1	35	9.2	-	-
25	4.0	25	6.0	40	10.0	0 (5)	0.0
30	4.1	30	6.5	45	10.7	5	1.2
35	4.7	35	7.0	52	11.0	11	3.2
45	5.0	48	8.2	55	11.5	15	3.3
50	5.1	52	9.0	60	12.0	25	5.2
55	5.3	60	9.1	-	-	35	6.2
60	5.5	-	-	0 (4)	0.0	45	7.2
-	-	0 (3)	0.0	6	1.2	55	8.1
0 (2)	0.0	5	2.0	13	3.0	60	8.3

Total pressure decrease Δp (scale divisions)

Rate of reaction R (scale divisions / 10mins.)

Δp	R	Δp	R	Δp	R	Δp	R
0.0	1.20	13.9	1.10	23.6	0.60	30.7	0.40
1.2	1.50	15.0	0.90	24.8	0.50	31.5	0.45
2.7	1.70	15.9	0.90	25.8	0.45	32.6	0.65
4.4	1.70	16.8	0.80	26.7	0.40	33.9	0.60
6.1	1.60	17.6	0.60	-	-	35.1	0.50
7.7	1.50	-	-	27.5	0.90	36.1	0.50
-	-	18.2	0.30	28.4	0.60	37.1	0.45
9.2	1.00	18.5	0.70	29.0	0.50	38.0	0.45
10.2	1.20	19.2	0.75	29.5	0.50	38.9	0.40
11.4	1.20	20.9	0.75	30.0	0.40		
12.6	1.30	22.4	0.60	30.4	0.30		

Total pressure decreaee Δp (scale divisions)

Total phosphoric oxide uptake of gas ΔP (scale divisions)

Δp	0.0	9.2	18.2	27.5	30.7	39.8
ΔP	0.0	5.5	14.6	26.6	33.6	41.9

The first most obvious fact arising from these results is that the effect of evacuation on the rate curves is duplicated by connecting in phosphoric oxide between periods of illumination. This would suggest either that the gaseous product formed in the primary reaction is involved in a secondary reaction :

$$A + mO_2 \rightarrow \text{products}$$

,this secondary reaction being a pressure increase reaction, or, that the removal of the gaseous product effects some change in the oxidised film, possibly a dissociation effect.

Secondly, the amount of phosphoric oxide gas uptake does not correspond to extrapolated values of 5 for ϕ, when the reaction was carried out in the presence of phosphoric oxide. (see fig.) This would suggest that the removal of a gaseous product is in fact increasing the susceptibility of the film to further oxidation.

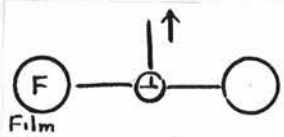
A decrease in the rate of reaction on leaving the film in contact with oxygen had been reported (29). No such effect appeared to exist in the presence of phosphoric oxide.

It is evident that, until the exact nature of the gaseous product or products was known, no satisfactory scheme for the reaction could be proposed. As a first stage it was shown that at least one gas could be isolated as a stable entity.

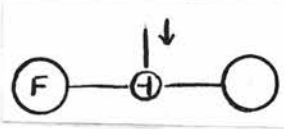
: (f) To show the possible isolation of a gaseous product from the oxidised chlorophyll film.

The stages are as follows :-

(1) Evacuate

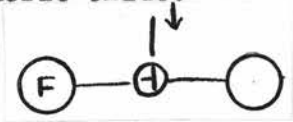


(2) Admit 100mm. of dry oxygen and carry out the normal reaction.

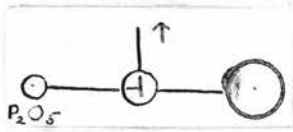


(3) Transfer part of the gas phase and reduce pressure in the gauge and jacket to 50mm.

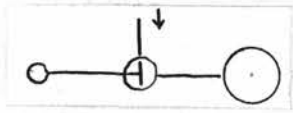
(4) Admit air to atmospheric pressure to F and replace F by a tube containing phosphoric oxide.



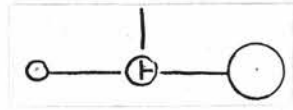
(5) Evacuate the phosphoric oxide for half an hour.



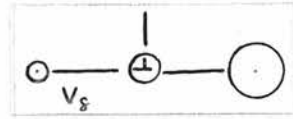
(6) Admit oxygen to a pressure of 50mm.



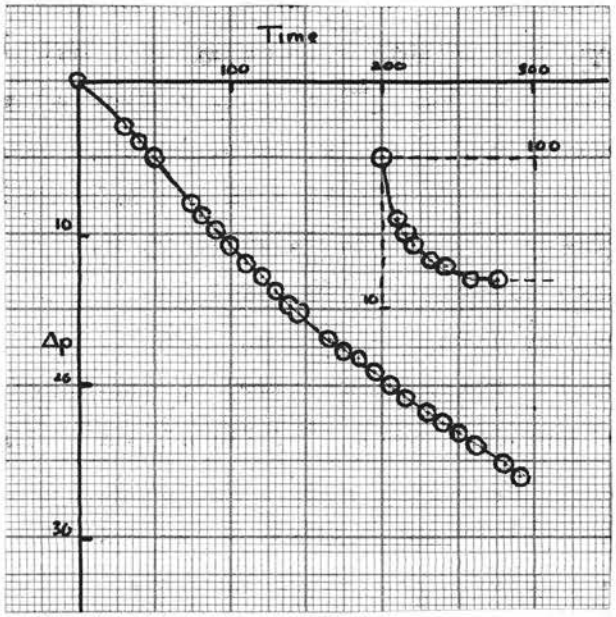
(7) Allow thermostating to come to equilibrium.



(8) Measure the uptake of gas by phosphoric oxide.



The procedure used in this experiment is indicated above. The normal reaction was carried out in one bulb, illumination stopped, and part of the gas phase transferred to a second evacuated bulb which was then isolated. The first bulb was then replaced by a tube containing phosphoric oxide which was evacuated for 30mins. before admitting oxygen to a pressure of 50mm. After allowing the system to come to equilibrium, the phosphoric oxide was allowed to come into contact with the gas phase sample and the uptake of gas measured.


Time
100
200
300
100
10
10
Δp
40
30

This experiment shows the possibility of actual isolation of a gaseous product, adsorbed by phosphoric oxide, from the reaction gas phase. A "blank" experiment carried out on oxygen, showed no uptake of any kind by the phosphoric oxide.

0.3gm TlBr. 3.78×10^{-6} gm. mole chlorophyll.

Total volume 90mls. Temperature 25° C.

Volume for uptake experiment (V8) 50.2mls.

Oxygen pressures as described above.

Time t (mins.). Pressure decrease Δp (scale divisions).

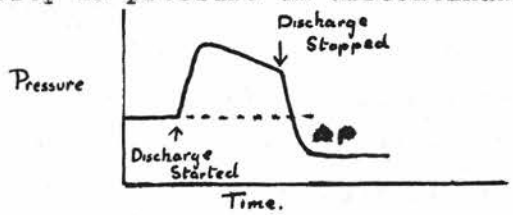
(a) Normal pressure decrease. (b) Phosphoric oxide uptake.

t	Δp	t	Δp	t	Δp	t	Δp
0 (a)	0.0	120	12.9	215	20.8	0 (b)	0.0
30	3.0	130	13.9	230	21.8	10	4.0
40	4.0	140	14.9	240	22.5	15	5.0
50	5.0	145	15.1	250	23.1	20	5.8
75	8.0	165	17.0	260	24.0	32	6.7
80	8.9	175	17.7	280	25.1	40	7.1
90	9.8	185	18.1	290	26.0	57	8.0
100	10.6	195	19.0	-	-	75	8.0
110	11.9	205	20.0	-	-	-	-

: (g) Effect of ozonisation on the gaseous reaction products.

Using a similar technique to the preceding, part of the gas phase, after a period of normal photo-oxidation, was transferred to a spherical soft glass vessel with two sealed-in platinum electrodes. The pressure changes occurring on sparking the gas sample were found to correspond exactly with a "blank" experiment. No evidence concerning the nature of the gas could therefore be deduced. The general characteristics of the pressure / time curves on sparking were, a very rapid rise in pressure on starting the discharge followed by a slow steady pressure decrease on continuation, and

a rapid drop in pressure on discontinuation of the discharge.

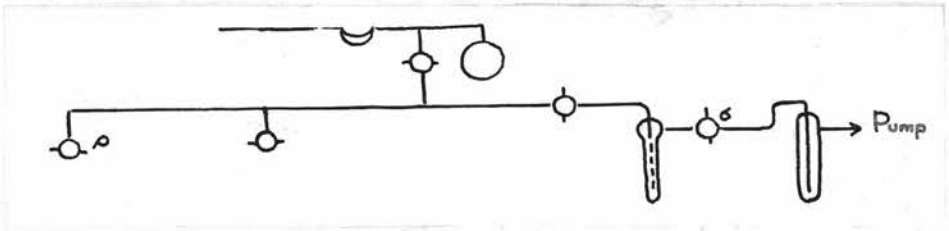


The characteristics of the curve were consistent with the reaction $3O_2 \rightarrow 2O_3$ together with a certain amount of heating accompanying the discharge. The net pressure change Δp is connected with the former, the rapid increase and decrease in pressure with the latter.

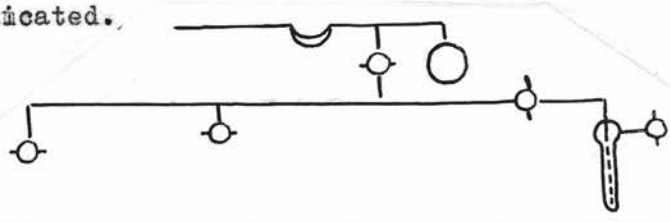
: Examination of the reaction products using a micro-distillation technique.

: (a) Attempt to detect a gaseous reaction product in a freezing-out experiment.

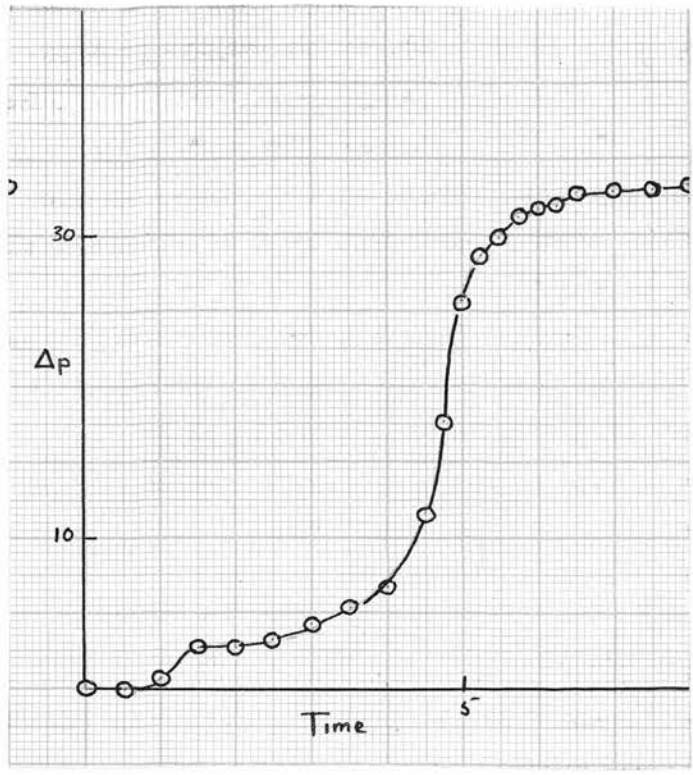
The "normal" reaction was carried out for a total pressure decrease of 12 scale divisions. The gas phase was then pumped slowly through a small trap in the system shown below.

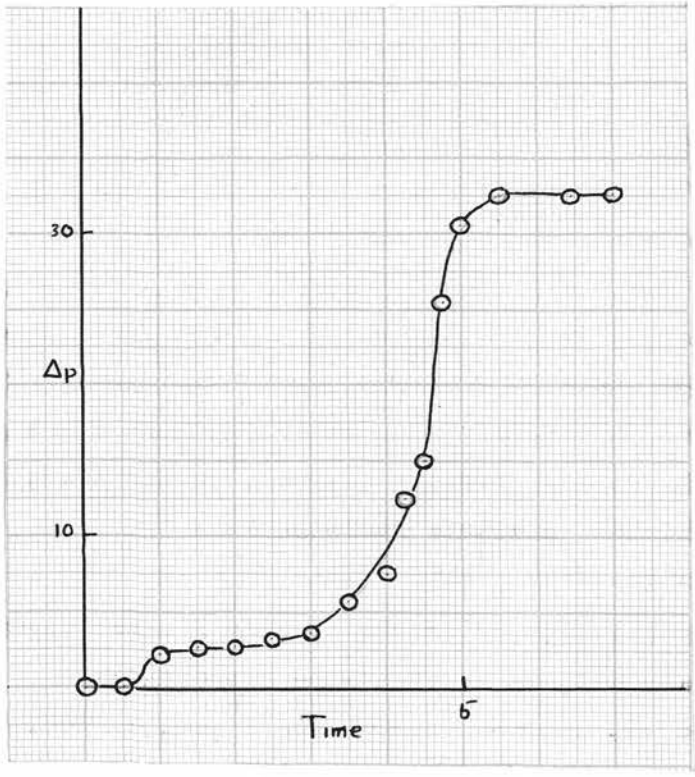


After closing taps ρ and σ, the liquid air was removed and the contents of the trap allowed to diffuse slowly into the system indicated.



Pressure measurements were made as this process took place and pressure/time curves obtained. A "blank" was carried out under identical conditions except that the illumination period was omitted.


30
ΔP
10
Time
5


30
ΔP
10
Time
5

0.3gms. TlBr. 5.2×10^{-6} gm. mole chlorophyll.
100mm. oxygen. Reaction volume 58.5mls.
Temperature 25°C. Gauge sensitivity 0.0230mm/ scale division.

The ratio of the reaction volume to the volume of the micro-distillation system was found by a simple expansion procedure to be :- 2.1:1.

Time t (minutes). Pressure increase Δp (scale divisions)

t	Δp	t	Δp	t	Δp	t	Δp
0.0	0.0	3.0	4.3	5.25	28.7	7.0	32.7
0.5	0.0	3.5	5.3	5.5	29.7	7.5	32.9
1.0	0.7	4.0	6.7	5.75	31.2	8.0	33.2
1.5	2.7	4.5	11.7	6.0	31.7	-	-
2.0	2.7	4.75	17.7	6.25	31.9	-	-
2.5	3.3	5.0	25.7	6.5	32.7	-	-

After freezing out the gases by replacing the liquid air round the trap, the distillation was repeated.

t	Δp	t	Δp	t	Δp	t	Δp
0.0	0.0	2.0	2.7	4.0	7.5	5.0	30.5
0.5	0.0	2.5	3.1	4.25	12.5	5.5	32.5
1.0	2.2	3.0	3.7	4.5	17.6	6.5	32.5
1.5	2.6	3.5	5.5	4.75	25.5	7.0	32.6

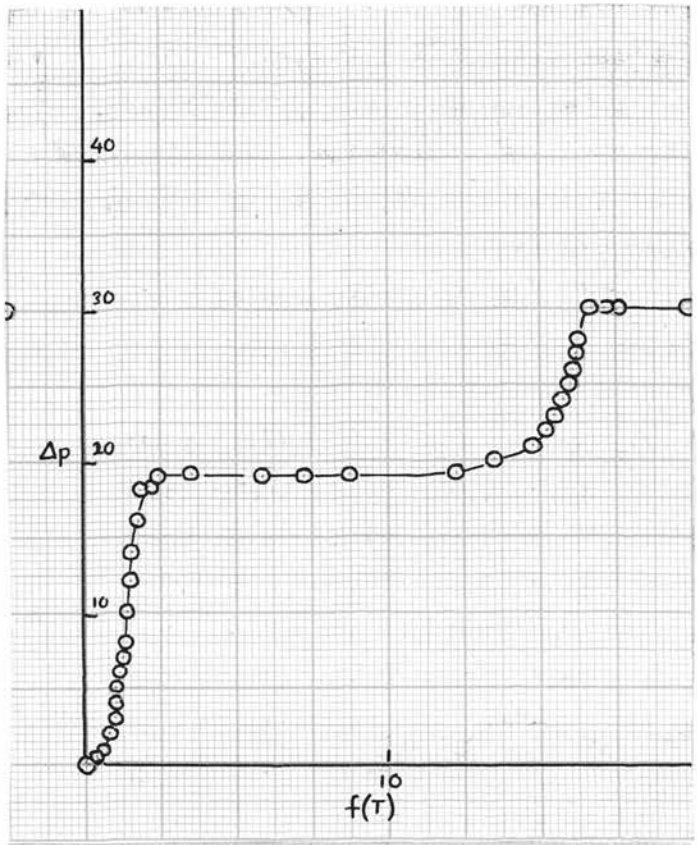
The "blank" experiment gave a pressure increase of 4.3 scale divisions under the same conditions.

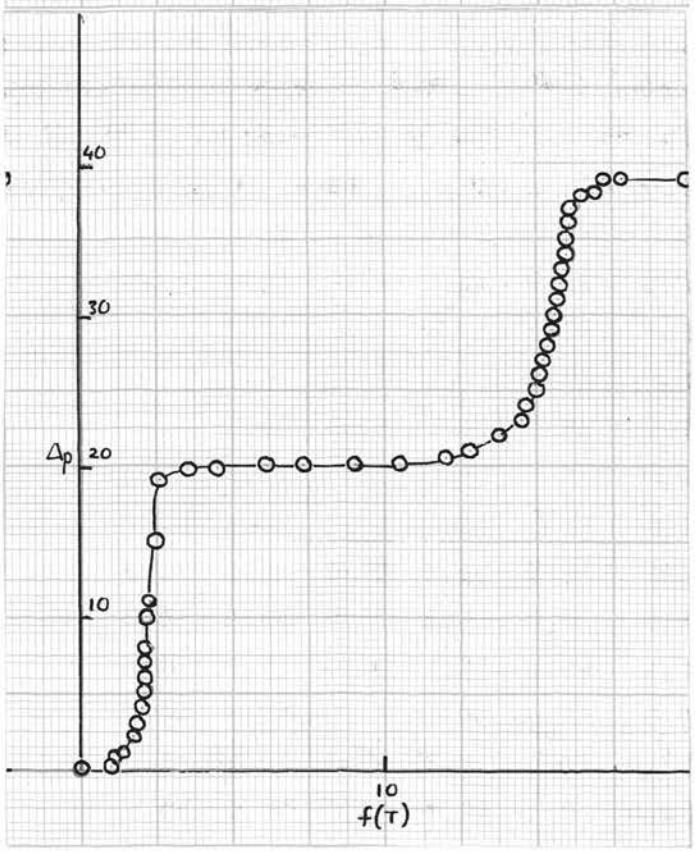
It is seen from these results that a double "step" exists in the distillation curve. This would suggest the presence of two gases in the reaction products. After subtracting the"blank" the second pressure increase amounts to 25.7 scale divisions. The ratio of the second pressure increase in the distillation curve to the "normal" pressure decrease is :

$$25.7 \times 2.1 / 12 = 4.5$$

: (b) Micro-distillation of carbon dioxide - water mixtures.

Two different methods of obtaining reproducible distillation curves were tried. The first involved the construction of pressure/time curves during the distillation.


40
30
Δp 20
10
10
f(τ)


40
30
Δp 20
10
10
f(τ)

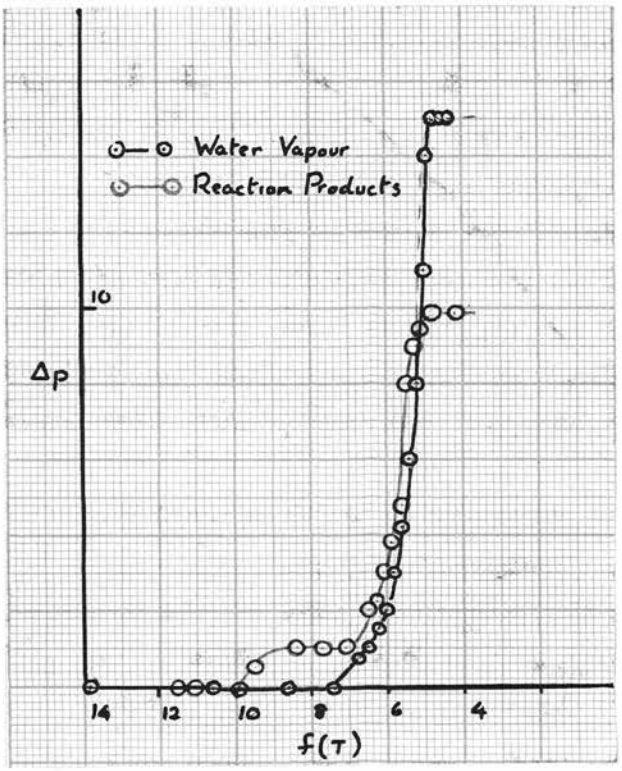
The second involved the use of a copper-constantan thermocouple and a galvanometer. In the second method, pressure was plotted against galvanometer reading, the latter being an undetermined function of temperature. The pressure / / f(temperature) curves were found to be more reproducible and two examples are included.

f(temperature) : f (galvanometer deflection).
pressure increase :Δp (scale divisions).

f	Δp	f	Δp	f	Δp	f	Δp
0.20	0.0	1.35	8.0	3.55	19.0	15.70	24.0
0.35	0.2	1.40	10.0	5.90	19.0	15.90	25.0
0.50	0.7	1.50	12.0	7.30	19.0	16.05	26.0
0.70	2.0	1.60	14.0	8.80	19.0	16.20	27.0
0.90	3.0	1.70	16.0	12.30	19.2	16.30	28.0
1.00	4.0	1.80	18.0	13.50	20.0	16.75	30.0
1.05	5.0	1.90	18.0	14.80	21.0	17.15	30.0
1.15	6.0	2.15	18.1	15.20	22.0	17.50	30.0
1.25	7.0	2.40	19.0	15.50	23.0	20.00	30.0

The mixture distilled was equivalent to 19 scale divisions of carbon dioxide and 18 scale divisions of water vapour. The carbon dioxide was completely recovered but some of the water vapour was adsorbed, only 11 scale divisions being recovered.

f	Δp	f	Δp	f	Δp	f	Δp
0.0	0.0q	2.3	11.0	12.8	21.0	15.9	33.0
0.8	0.2	2.4	11.0	13.8	22.0	16.0	34.0
0.9	0.5	2.5	15.0	14.5	23.0	16.0	35.0
1.3	0.9	2.6	19.0	14.7	24.0	16.1	36.0
1.7	2.0	2.8	19.0	15.0	25.0	16.2	37.0
1.8	3.0	3.6	19.7	15.1	26.0	16.5	37.8
2.0	4.0	495	19.8	15.2	27.0	16.6	38.0
2.1	5.0	6.2	20.0	15.4	28.0	16.9	38.0
2.1	6.0	7.3	20.0	15.5	29.0	17.2	39.0
2.2	7.0	9.0	20.0	15.6	30.0	17.8	39.0
2.2	8.0	10.5	20.1	15.7	31.0	18.0	39.0
2.2	10.0	12.0	20.5	15.8	32.0	20.0	39.0


Water Vapour
Reaction Products
10
Δp
14
12
10
8
6
4
f(T)

In the second case 20 scale divisions of carbon dioxide were completely recovered and adsorption effects again led to a discrepancy in the amount of water vapour recovered, only 19 scale divisions being recovered out of 25 originally present.

From these distillation curves it would seem that each gas distils off at a specific value of f(T), which is of course a function of temperature, and that quantitative estimations of small amounts of carbon dioxide can be made by this method. The quantitative estimation of water vapour by this method is not so satisfactory and indeed the effect of adsorption on ϕ in the normal reaction cannot be neglected.

(c) Examination of the reaction products.

The normal reaction was carried out and the reaction gas phase pumped out slowly through the micro-trap. The pressure/f(T) curve obtained on distillation was compared with a water vapour standardisation curve carried out under identical conditions.

0.3gm TlBr. 5.2×10^{-6} gm mole chlorophyll.

100mm. oxygen. Temperature 25° C.

Reaction volume 58.5mls. Gauge sensitivity 0.0230mm/scale div.

f A	Δp	f	Δp	f B	Δp	f	Δp
13.9	0.0	5.6	6.0	11.5	0.0	6.1	3.0
10.6	0.0	5.2	8.0	11.1	0.0	5.9	3.8
8.6	0.0	5.0	11.0	9.9	0.0	5.6	4.8
7.4	0.0	4.9	14.0	9.5	0.5	5.4	8.0
6.7	1.0	4.8	15.0	8.4	1.0	5.2	9.0
6.2	1.5	4.6	15.0	7.7	1.0	5.1	9.5
6.0	2.0	4.5	15.0	7.1	1.0	4.9	9.9
5.8	3.0	-	-	6.5	2.0	4.7	9.9
-	-	-	-	6.3	2.2	4.2	9.9

A is the water vapour standardisation curve, B, the distillation curve for the frozen-out products of the "normal" reaction.

It would appear from the similarity between the curves that one of the products of the reaction is water vapour. A "blank" carried out on oxygen gave negative results. It is noticeable that, in spite of the marked similarity of the distillation curves, a dissimilarity exists at low temperatures. The small initial "step" in the curve B will be discussed later.

: (d) Correlation of the amount of water vapour produced in the normal reaction with the normal pressure decrease.

Attempts at correlation were a complete failure, partly because of discrepancies caused by the adsorption of water vapour, and partly, it is thought, due to a dissociation pressure effect similar to that causing the non-reproducibility of ϕ in the presence of phosphoric oxide.

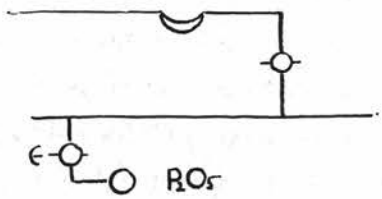
The values obtained for a normal pressure of 10scale divisions at the beginning of the normal reaction, are as shown :- 13.8, 70, 23.

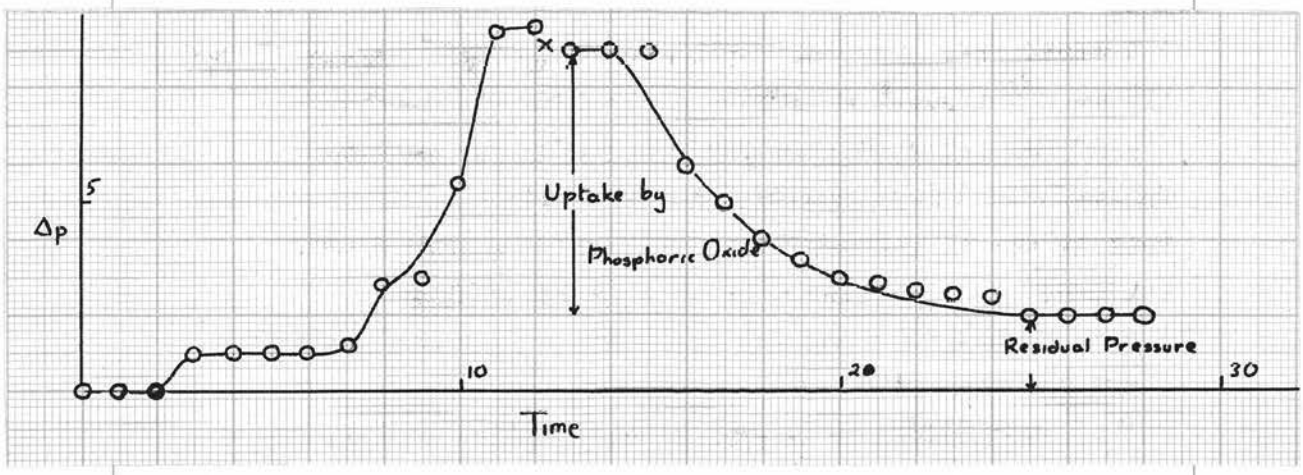
A value of 21 corresponds to a unit ratio.

: (e) Presence of other gases in the reaction products.

Although a small rise in pressure was usually detected in micro-distillation experiments before water vapour distilled off, it was not found possible to obtain a curve exactly similar to the distillation curve for carbon dioxide. That there was a second gas in the distillate was shown by the following experiment :-

During the evacuation preceding a period of normal reaction a small side tube containing phosphoric oxide, attached as shown, was also pumped out. The tap ϵ was then closed and after a period of normal reaction a micro-distillation of the products was carried out into the reaction space. On opening tap ϵ the water vapour present in the distillate was taken up rapidly but a small residual pressure of some unknown gas or gases remained.


P_2O_5


Δp
5
Uptake by
Phosphoric Oxide
Residual Pressure
10
20
30
Time

Time t (minutes). Pressure change Δp (scale divisions).

t	Δp	t	Δp	t	Δp	t	Δp
1	0.0	8	2.8	15	9.0	22	2.7
2	0.0	9	3.0	16	6.0	23	2.6
3	1.0	10	5.5	17	5.0	24	2.5
4	1.0	11	9.5	18	4.0	25	2.0
5	1.0	12 X	9.6	19	3.5	26	2.0
6	1.0	13	9.0	20	3.0	27	2.0
7	1.2	14	9.0	21	2.9	28	2.0

At the point "X" the tap was opened.

: Attempted Photo-reduction of chlorophyll.

Oxalic acid is found to photo-oxidise on thallous bromide (30) with the formation of carbon dioxide and water. In view of the fact that chlorophyll also photo-oxidises, it was thought possible that the photo-reaction of an oxidised chlorophyll film with anhydrous oxalic acid might well result in the regeneration of the chlorophyll film. Attempts to find such an effect failed. The results obtained are summarized below.

(a) A mixed film of chlorophyll and oxalic acid was illuminated
  (1) in the absence of oxygen :- no pressure change.
  (2) in the presence of oxygen :- a pressure increase followed by a pressure decrease occurred.
  This is due to the rapid oxidation of the oxalic acid followed by the slower oxidation of chlorophyll.

(b) A film consisting of oxidised chlorophyll and oxalic acid was illuminated
  (1) in the absence of oxygen :- no pressure change.
  (2) in oxygen :- a pressure increase occurred.
  This is due to the normal oxidation of oxalic acid.

It would seem from these results that no oxidation of oxalic acid by illumination of a mixed film of oxidised chlorophyll and oxalic acid is possible in the absence of oxygen. A possibility in this reaction is the participation of chlorophyll as a photo-sensitiser.

Discussion.

Most of the work carried out in this research was carried out on copper-stabilised chlorophyll. A brief discussion of the effect of this part substition is therefore necessary. Copper replaces magnesium very readily in the chlorophyll molecule, so readily in fact, that this precludes the use of copper in industrial extraction plant (31), where relatively pure chlorophyll is required. It was found by previous workers that the presence of copper seemed to alter only the reaction rate of the photo-oxidation without marked effect on the essential features. In other words, the copper-substituted molecule acts as an anticatalyst in the reaction. In addition to the stabilising effect towards photo-oxidation produced by a small percentage of copper-substituted molecules, the absorption spectrum of solutions is shifted towards the red and the marked red fluorescence associated with chlorophyll is destroyed or markedly weakened (32). The effect is one peculiar to the chlorin ring system, for, in the oxidation of the simpler tetraphenyl chlorins(33) exactly the same effect has been observed. It has been suggested that the presence of the paramagnetic copper atom in a molecule is sufficient to break down the transition prohibition to which a triplet state molecule owes its relative stability and prolonged lifetime (34). That is to say, that a molecule containing copper although it may reach the triplet state, will readily lose its energy by some unspecified degradative process. This theory agrees with the non-phosphorescence of copper tetraphenyl chlorin (35)

In solution chlorophyll shows self-quenching of fluorescence, i.e. a slight decrease in fluorescence with increased concentration. It has been suggested (36) that this is due, not to dimer formation or collision quenching but to a resonance exchange of excitation energy. If such an exchange can occur in solution, it seems reasonable to assume that in

the solid state also, this can occur, so that, if Chl represents a molecule which has absorbed energy, this energy is equally available to adjacent molecules for chemical reaction.

The lattice $\overset{*}{\text{Chl}}$ Chl Chl Chl Chl

where is the site of the excitation energy, behaves in fact, like the lattice Chl Chl Chl Chl Chl Chl

where the excitation energy is not localised.

The inhibiting effect of the copper chlorophyll on the photo-oxidation may be explained as a defect in the lattice whereby the energy available for oxidation "drains" away.

$$\overset{*}{\text{Chl}} \rightarrow \text{Chl} \rightarrow \text{Chl} \rightarrow \text{Chl} \rightarrow \text{Chl} \rightarrow \text{Chl(Cu)}$$

$\sim$ internal degradation.

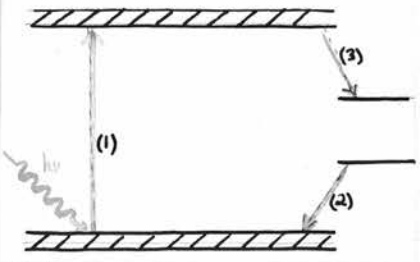
Although it was found convenient to use partly copper-substituted material, because of the possible rapid deterioration of samples of pure chlorophyll, the slow reaction rates on a glass surface rendered investigations rather tedious (37). The sensitising effect of thallous bromide on the photo-oxidation provides a convenient method of speeding up investigations (38) and has generally been used by workers in this field. Thallous bromide is similar to silver bromide in being a photo-conductive solid. The sensitising effect may be due to two causes:-

(a) Thallous bromide may itself absorb light quanta and transfer the energy to the chlorophyll.

(b) It may serve as a convenient communication system between a light-absorbing molecule of chlorophyll and a molecule capable of photochemical reaction, that is, it may form an extension of the chlorophyll "lattice" and may amplify the interchange of energy mentioned above.

Some overlap between the "reactive" energy level of the chlorophyll molecule, probably a triplet state, and the conductive level in thallous bromide is necessary for the second of these effects to occur. The process is represented

as an extension of the interchange of energy in the chlorophyll lattice, previously mentioned. The first process is represented schematically below and is probably the more important since chlorophyll desensitises thallous bromide plates, that is, prevents latent image formation (39).



Conduction band of thallous bromide.

Excited (reactive) state of chlorophyll.

Ground state of chlorophyll.

Ground level of thallous bromide.

(1) On illumination, an electron is raised to the conduction band in thallous bromide.

(2) Chlorophyll loses an electron to the thallous bromide lattice.

(3) Chlorophyll gains an electron from the conduction band of thallous bromide with the formation of an excited chlorophyll molecule, probably in the triplet state.

Both processes seem feasible and, in addition, the possibility of an ionic reaction between $Chl^{\oplus}$ formed as in process (2) above, and a negatively charged oxygen ion $O_2^{\ominus}$ formed by a process similar to (3), cannot be excluded.

These hypotheses apply only to the initial reactions in the sequence. Until the initial energy transfer is investigated thoroughly, it seems pointless to discuss the later reactions with reference to the thallous bromide lattice.

The precise mechanism for the oxidation of chlorophyll is of interest not only as an example of a photo-degradative process, but also because of its possible participation in the photosynthetic processes in the living plant. Whether chlorophyll acts as a reactant or as a photosensitiser in this process, has never been conclusively demonstrated. Kautsky (40) attempted to correlate fluorescence in the living leaf with the photo-oxidation of a chlorophyll complex. It is interesting to note that, whereas in the photosynthetic

process carbon dioxide and water are reactants and oxygen a product, the reaction sequence in the present case involes oxygen as a reactant and carbon dioxide and water as products.

The mechanism originally evolved for the photo-oxidation of chlorophyll was :-

$$Chl + h\nu \quad Chl^{\cdot\cdot}$$
$$Chl^{\cdot\cdot} + O_2 \rightarrow Chl O_2 r$$
$$Chl O_2 r \rightleftharpoons Chl + O_2$$
$$Chl O_2 r \xrightarrow{h\nu ?} Chl O_2 i$$

(page 6.)

The mechanism we are now forced to adopt is much more complex, involving the reaction products water and carbon dioxide.

One of the first objections to the simple mechanism was the fact that, in the presence of absorbents, the value of ϕ rose markedly above unity. Such values of ϕ were extremely scattered; in the case of phosphoric oxide values for ϕ of 5.3, 4.0 and 2.7 were found, and in a vessel of greater volume 1.75 and 2.05. In the cases where ϕ was ~2, the "reversibility" was not destroyed, i.e. on evacuation an increased rate of reaction was obtained. This would suggest, since "reversibility" is characteristic of the normal reaction, that, in these cases, although removal of some gaseous product was taking place to account for the increased value of ϕ, no alteration in the structure of the oxidised film had occurred. This might well entail the existence of some critical partial pressure of one of the reaction products, below which further degradation of the film could occur. In the presence of both phosphoric oxide and soda-lime the value of ϕ rose above 5.

The experiments with the three-way tap, fully described in the experimental section, were of great value, not only as giving an indication of the true reaction mechanism, but also in eliminating certain reaction sequences as possibilities. This technique led to the following results :-

(a) The effect of absorbents is not a pre-treatment effect, i.e. the increased pressure decrease obtained in the presence of absorbents is not due to a sequence of reactions such as :-

$$Chl \rightleftharpoons A + X \qquad (1)$$

$$X + P_2O_5 \rightarrow P_2O_5(X) \qquad (2)$$

$$A + 5O_2 \rightarrow Chl(5O_2) \qquad (3)$$

but is in fact due to the uptake of some gaseous product formed during the reaction(page 24).

(b) The gaseous products are not further photo-degraded (page 26).

(c) It is possible to isolate a gaseous reaction product which is taken up by phosphoric oxide (page 30).

(d) The effect of phosphoric oxide being connected into the gas phase at various stages during the reaction is exactly similar to the effect of evacuation on the normal reaction, i.e. an increase in rate occurs (page 30).

(e) It is possible to correlate the amount of normal pressure decrease with the amount of gaseous reaction product taken up by phosphoric oxide. The uptake of gas by phosphoric oxide appears to be equal to the normal pressure decrease, at least in the early stages of reaction (page 30).

Attempts to identify the reaction products included an unsuccessful ozonisation experiment. Micro-distillation techniques indicated the presence of water vapour and possibly carbon dioxide as resultants in the reaction.

Before discussing a possible reaction mechanism, some results, obtained subsequent to the experimental work described in this thesis, will be outlined (41).

(a) The presence of water vapour, carbon dioxide and acetone vapour in the reaction gas phase, has been indicated by various methods including a more detailed analysis of the micro-distillation curves. The relative amounts of these products varied considerably, both in relation to each other and to the amount of normal reaction. A typical analysis of the

condensable material when the normal pressure decrease amounted to 30% of the extrapolated value was :-

water vapour	90%
carbon dioxide	4%
acetone	6%

The ratio water vapour : normal pressure decrease was found to be 2 : 1 in the initial stages of the reaction but was found to rise as the reaction proceeded, a ratio of 3.5 : 1 being found at a later stage. The ratio carbon dioxide : water vapour was also found to ride during the reaction.

(b) A temporary removal of condensables from the gas phase by means of a side tube cooled in liquid oxygen, produced the same effect as evacuation and the irreversible removal of water vapour by connecting in phosphoric oxide, viz. an increase in reaction rate was obtained, even though the condensables were allowed to distil back into the reaction vessel prior to re-illumination. This implies that the dissociation process postulated as indirectly responsible for the increased rate is almost completely irreversible.

(c) At low oxygen pressures in the presence of phosphoric oxide the rate of the reaction did <u>not</u> decrease to zero at a finite gas pressure as does the normal reaction rate.

The reaction scheme shown below may be considered generally in relation to the experimental results :-

$$Chl + h\nu \rightarrow Chl^{*} \qquad (1)$$

$$Chl^{*} + 2O_2 \rightarrow AO_2 + H_2O \qquad (2)$$

$$AO_2 \rightleftharpoons BO_2 + H_2O \qquad (3)$$

$$AO_2 + 4O_2 \xrightarrow{h\nu} DO_2 + 3H_2O + CO_2 \qquad (4)$$

$$BO_2 + 4O_2 \xrightarrow{h\nu} DO_2 + 2H_2O + CO_2 \qquad (5)$$

AO_2, BO_2, and DO_2 are solid phase degradation products. AO_2 is in equilibrium with BO_2 and water vapour. Reactions (2), (4) and (5) are themselves multi-stage reactions.

The normal reaction at high oxygen pressures, is represented by reactions (1), (2) and (4), in which 6 oxygen

molecules are taken up with the production of 4 molecules of water vapour and one of carbon dioxide. It seems probable that in the early stages of the normal reaction only (1) and (2) are operative. In all cases the normal reaction should take place with ϕ equal to unity. Values of ϕ greater than unity might be expected because of the adsorption of water vapour by glass surfaces. At low oxygen pressures the rate of reaction has been found to approach zero at a finite oxygen pressure (42). In view of the effect of phosphoric oxide on this feature of the reaction, and since, even below the apparent "dissociation pressure", a slight pressure decrease had been observed, it is suggested that this feature of the reaction is due, not to the dissociation pressure of an organic peroxide of the anthracene type, but to the water vapour pressure of compound AO_2. On evacuation of the system, connecting in phosphoric oxide or on freezing out condensibles, after a period of normal reaction, an increased rate of reaction is obtained on re-illumination. This is due to the formation of compound BO_2 and its subsequent photo-oxidation, comparable to reaction (2) in quantum effeiciency. The increased rate is not a reversibility effect.

If the reaction is carried out in the presence of absorbents, the value obtained for ϕ will depend on how efficiently water vapour is removed from the gas phase. Inefficient removal of water vapour will give $\phi = 2$, since BO_2 wil not be formed and (5) will not come into operation until a late stage in the normal reacrion. The correlation between phosphoric oxide uptake (water vapour) and normal pressure decrease to give a unit ratio in the early stages of the reaction was found experimentally.

Reaction (3) is responsible for (a) an induction period before the apparent reaction velocity reaches its maximum, for the build-up of water vapour by the dissociation process is directly opposed to the normal pressure decrease: (b) the

fact that an "apparent" dissociation pressure could be measured: (c) the anomalies in the phosphoric oxide experiments due to difficulties in obtaining a reproducible rate of absorption of water vapour from the gas phase. If the rate of absorption is greater than that of reaction (1) $\phi = 5$, if less $\phi = 2$.

Reaction (4) is a late participant in the normal reaction and accounts for the formation of carbon dioxide. Reaction (5) is the reaction responsible for the increased rate obtained on evacuation. Although assumed to be comparable to (4) in quantum efficiency, reaction (5) is a pressure decrease reaction so that its participation gives an overall increased rate of pressure decrease. The comparatively late participation of reaction (4) in the overall normal reaction would account for the relatively small amounts of carbon dioxide found during the early stages of the normal reaction.

The complicating effect of what appears to be the slow desorption of acetone from the film during the course of the reaction need not be emphasised. It is thought however, that this may have partly compensated for the adsorption of water vapour on the glass surface in that unit ratios for ϕ have been consistently found by previous workers.

Little work was carried out on the carotenes, but again evidence for gaseous reaction products was obtained. The attempted photo-reduction of oxidised chlorophyll by means of oxalic acid was too complex to yield any useful results without a detailed study of reaction rates. The photo-oxidation of oxalic acid itself on thallous bromide (43) is a sufficiently rapid reaction to mask any subsidiary processes to a large extent.

The ultimate test of the validity of this mechanism depends on the isolation and identification of the compounds AO_2, BO_2, and DO_2. On the present micro-scale this would appear to be rather difficult and an extension to the macro-scale is required. The reaction mechanism postulated involves

the uptake of six molecules of oxygen with the formation of four molecules of water vapour and one of carbon dioxide. It is interesting to consider at what points in the chlorophyll molecule oxidative reaction can take place.

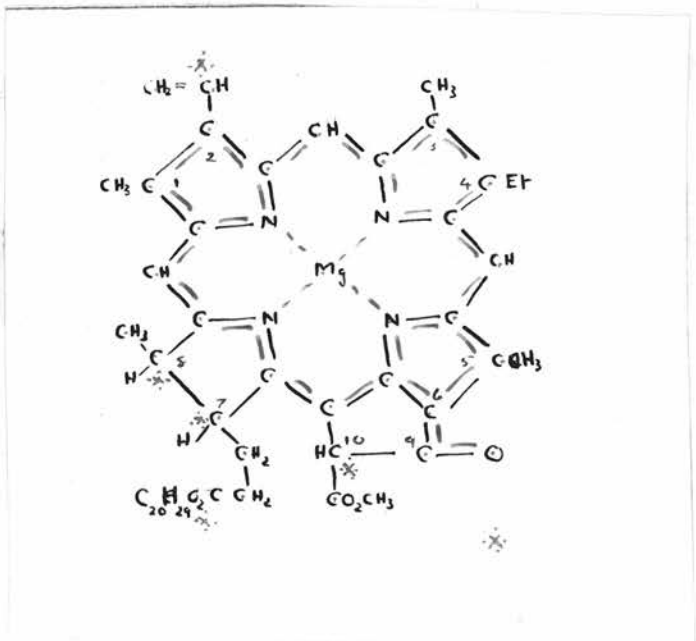
One complication must be mentioned :- the hydration of chlorophyll. Hanson (44) found by a study of surface films that chlorophyll took up a variable amount of water of hydration. The complicating effect on interpreting results, in a reaction where water vapour is a reaction product, need not be stressed. The possibility that the hydrated product of reaction (1) is not susceptible to further oxidation agrees reasonably well with the remainder of the mechanism.

$$\text{Chl(hydrated)} + 2O_2 \rightarrow AO_2\text{(hydrated)} + H_2O$$

$$AO\text{(hydrated)} \rightleftharpoons BO_2 + H_2O$$

If the hydration of chlorophyll is a complicating factor, then the dissociation pressure of the chlorophyll hydrate must be very small, since evacuation of the system to a pressure of 10^{-6} mm. does not alter the essential features of the subsequent <u>normal</u> reaction (45).

The main reactive centres in the chlorophyll molecule are as shown below.



(1) The hydrogen atom at C_{10} may be attacked with the formation of a peroxide :-

$$C-H + O_2 \rightarrow CO-OH$$

To such a reaction have frequently been attributed such effects as the Molisch phase test (46). Such a reaction would account for the uptake of one molecule of oxygen. However allomerised chlorophyll, in which a C_{10} position is regarded as already oxidised, gives a value of unity for ϕ in the <u>normal</u> reaction.

(2) The hydrogen atoms attached to C_7 and C_8 are also vulnerable to oxidation e.g. the simpler chlorins, on photo-oxidation give the corresponding porphins :-

$$\begin{matrix} CH \\ | \\ CH \end{matrix} + O_2 \rightarrow \begin{matrix} C \\ \| \\ C \end{matrix} + H_2O_2$$

Such a reaction would account for the uptake of one molecule of oxygen, but not, by itself, for any of the subsequent pressure changes. The formation of hydrogen peroxide in the reaction is a complicating factor. Its rapid attack on the remainder of the chlorin nucleus could lead to the formation of other oxidisable centres.

(3) The reaction of the vinyl group :-

$$-CH=CH + O_2 \rightarrow -\overset{\overset{O}{\|}}{C}-CH_3$$

would also account for the uptake of one molecule of oxygen.

(1), (2) and (3) are the most obvious possibilities but, by themselves, they account only for the uptake of three molecules of oxygen. To account for the uptake of six molecules of oxygen, either the phytol side chain, which contains a double bond may be oxidised, or simultaneously with the initial photo-reaction a complex fission of the molecule into non-volatile fragments capable of further photo-oxidation, may occur.

It is suggested that future investigations of the photo-degradation of chlorophyll in the solid state should be directed along three main lines.

(a) So far, investigations have been carried out mainly on copper-stabilised samples of chlorophyll, due to the possible rapid deterioration of unstabilised samples. By the use of low-temperature storage in addition to the exclusion of light it is thought that pure chlorophyll samples would remain intact for a period sufficient for investigations to be carried out on them. Chlorophylls α and β should be investigated separately since one may sensitise the reactions of the other.

(b) The reactions of the simpler chlorin compounds in similar conditions might well give an indication of the possible fission of the molecule during reaction.

(c) An attempt to identify the products of photo-oxidation, possibly by the use of chromatographic separation techniques, should be made since no complete mechanism can be built up without this information.

Summary

A mechanism has been derived for the photo-oxidation of partly-copper-substituted chlorophyll in the solid state, based on several consecutive reactions involving the production of water vapour, carbon dioxide, and three solid degradation products, one of which has a dissociation pressure of water vapour. The reaction scheme gives an explanation of most of the experimental data. It is thought that the modifications of apparatus adopted during this research should prove extremely useful in the investigation of the solid phase degradations and photo-degradations of dyestuffs generally.

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